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Fatty acid composition of oils, their oxidative, flavor and heat stabilities and the resultant quality in foods

Caiping Su
Iowa State University

Fatty acid composition of oils, their oxidative, flavor and heat stabilities and the resultant quality in foods

by

Caiping Su

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Program of Study Committee:
Pamela White, Major Professor
Daniel Nettleton
Earl Hammond
Terri Boylston
Tong Wang

Iowa State University

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Iowa State University

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Caiping Su

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Committee Member

Signature was redacted for privacy.

Committee Member

Signature was redacted for privacy.

Committee Member

Signature was redacted for privacy.

Committee Member

Signature was redacted for privacy.

Major Professor

Signature was redacted for privacy.

For the Major Program

DEDICATION

To my parents

who raised me in a family rich in love and strong in bond.

The degree

is for the one you never had a chance to attain.

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ABSTRACT

Soybean oil (SBO) is an oxidatively unstable oil, largely because of the high concentration of linoleic acid (18:2) and linolenic acid (18:3). The unsaturated fatty acids, oleic acid (18:1), 18:2, and 18:3 in SBO oxidize in a ratio of 1: 10.3: 21.6. To improve oxidative and flavor stability, the SBO may be hydrogenated to reduce the concentration of PUFA (and increase the saturated FA); however, *trans* fatty acids (*t*FA) are formed and saturated fatty acids are increased during this process. There are health concerns over the consumption of a diet high in *trans* FAs and high in the ratio of saturated fatty acids to PUFA. Lowering the 18:3 content to a level similar to that obtained by partial hydrogenation, but without *trans* formation and increasing saturation has been objectives of plant breeders. A diet high in monounsaturated has been shown to help reduce health risks. Elevating 18:1 in seed oils has become more and more common.

The objectives of this study were to 1) study the effects of two low levels of 18:3 concentration (~1.0% and 2.2%) on the oxidative and flavor stabilities of SBO and 2) determine the optimum percentage of oleic acid (OA) in six SBOs (including high-oleic SBO (79%OA), conventional SBO(control), three blended oils containing 36.9%, 50.7%, and 64.7% OA, abbreviated as 37%OA, 51%OA, and 65%OA, respectively, and a low-linolenic (LL, contained 1.4% linolenic acid) SBO, to obtain maximum frying stability while retaining good flavor potential.

In general, results of the storage study suggested that the SBO containing 1.0% 18:3 had generally significant better oxidative and flavor stability during storage at 21 and 32°C

than did SBO contained 2.2% 18:3. Results of the frying study suggested that the order of oxidative stability of the six oil treatments was: 79%OA > 65%OA > 51%OA > LL ≥ 37%OA > Control, and that the order of flavor stability and eating quality of foods fried in them was: LL ≥ 79%OA > 65%-OA > 51%-OA > 37%-OA > Control.

These findings should help soybean breeders more precisely decide compositional targets to produce SBO that have desirable properties.

GENERAL INTRODUCTION

Since the 1980s, public health advice on diets for prevention of coronary heart disease (CHD), and therapeutic diets for the treatment of these cardiac patients, has recommended the consumption of low-fat diets with high polyunsaturated (PUFA) to saturated fatty acid (SFA) ratios. As a result, the consumption of vegetable oils, such as soybean oil (SBO), has increased over animal fats known to contain cholesterol and high amounts of SFAs (1). However, SBO has poor oxidative stability and its flavor deterioration presents challenges to the food oil industry.

The process of catalytic hydrogenation of vegetable oils was discovered in 1897 to reduce the PUFA and to improve flavor stability, versatility and performance of vegetable oils in salad dressings, during cooking, in deep-fat-frying, and for margarines, shortenings, and other baking and snack food applications (2). However, another important factor in hydrogenation is the formation of positional and geometrical isomers. Formation of trans isomers is rapid and extensive (3). In the United States, hydrogenated soybean oil (HSBO) is the primary dietary source of fatty acid isomers, because about 90% of the hydrogenated vegetable oil produced is HSBO (4). The estimated *trans* FAs intake by typical U.S. consumers is 11.1 to 27.6 g/person/day (5). A comprehensive review concluded that *trans* FAs consumed at 4.0% or more of total calories may raise plasma lipid levels (6). Because of health concerns over the presence of *trans* FAs in our diet, modifying fatty acid composition of SBO to improve its oxidative and flavor stability as obtained by hydrogenation, but without *trans* formation, has been an objective of plant breeders.

Studies have shown that the oxidation rate of oleic acid (OA, 18:1) is much slower than that of the PUFA, linoleic (18:2) and linolenic (18:3), which oxidize quickly and are the major contributors for the poor stability of SBO (7, 8). Therefore, trends in oilseed breeding have been to create oilseed crop producing specialty oil in which a particular fatty acid predominates or diminishes with its own targeted industrial application and market value (9, 10). For example, (1) a low-saturated oil, such as low-palmitic acid SBO, is aimed at meeting consumers' dietary needs for less saturated fatty acids for better health; (2) a high-saturated oil, such as high-stearic or high-palmitic acid SBO, has improved stability and is suitable for making low to zero *trans* margarine and shortening for health-conscious consumers; (3) a low-linolenic aicd oil is aimed at increasing flavor and oxidative stability; (4) a high-oleic acid oil (low-saturated and low-linolenic acid) oil is aimed at improving both stability and a healthful image.

The overall objective of current study was to determine the oxidative, flavor and heat stabilities of SBO with modified FA composition and the resultant quality in foods processed using SBO with modified fatty acid compositions through conventional plant breeding. The long-term goal is to aid oil-seed breeders, food-product manufacturers, and consumers through the development of better and more healthful vegetable oils. These goals have been accomplished by completing two separate, but related, projects.

In the first project, the objective was to evaluate the effects of 18:3 concentration, combined with TBHQ addition, temperature, and storage time, on the oxidative and flavor stabilities of SBO during storage under light. In the second project, the frying stability of six SBO treatments including Control (conventional SBO containing 21.5% OA), LL (low-linolenic acid SBO containing 1.4% 18:3 and 25.3% OA), three blended oils of Control with

high OA SBO at different ratios to result in oils of 37% OA, 51% OA, 65% OA, and 79%OA (high OA SBO containing 79.0% OA), respectively, were studied. One objective of this second project was to determine the optimum percentage of oleic acid (OA) in SBOs that could be achieved by blending high-oleic (HO, 79% OA) and conventional SBO (21.5% OA) to obtain maximum frying stability while retaining good flavor potential and quality in fried food. It is a common belief that the blended oils can be only as stable as the "poorest" oil. A second objective was to determine the impact of blending a relatively unstable control SBO with a highly stable HO SBO on the frying stability of the blended oils.

Dissertation organization

This dissertation is composed of a general introduction, a literature review, four papers, and a general conclusion. Discussed in the literature review are the pathways of lipid oxidation, lipid oxidation products and their significances, factors affecting fat and oil stability and quality, measures to improve oil and fat stability and quality including hydrogenation, modification of fatty acid composition of oil-seed through plant breeding and use of antioxidants. The first of the four papers was published in the Journal of the American Oil Chemists' Society in February of 2003. The second was submitted to the journal and is being reviewed. The last two papers will be submitted to the same journal for publication soon. Following the fourth paper are general conclusions and a list of references cited in the general introduction and literature review.

LITERATURE REVIEW

Lipid Oxidation

Autoxidation Extensive work has been done to clarify the mechanism of lipid oxidation and it is widely agreed that "autoxidation" is the most common reaction involved (11, 12, 13, 14). Autoxidation is a spontaneous reaction catalyzed by light, heat and metals and involving the incorporation of molecular oxygen with unsaturated fatty acids to produce hydroperoxides. Autoxidation is, in most instances, a free radical (a free radical is a molecule with unshared valence electron) chain reaction that includes three steps: initiation, propagation and termination (15, 16).

Initiation: $RH \rightarrow R\bullet + H\bullet$ Eq. 1

Or: $ROOH \rightarrow RO\bullet + \bullet OH$ Eq. 2

$RO\bullet + RH \rightarrow ROH + R\bullet$ Eq. 3

In the initiation step, the formation of the first free radicals may take place by thermal dissociation (thermolysis), by hydroperoxide decomposition, by metal catalysis and by exposure to light (photolysis, initiated by UV-catalyzed decomposition of peroxides and hydroperoxides) with or without photosensitizers. An induction period (time before rapid oxidation occurs) is usually observed in lipid oxidation at the very beginning when the oil is subjected to oxidative stress to create the very first free radicals.

Propagation: $R\bullet + O_2 \rightarrow ROO\bullet$ Eq. 4

$ROO\bullet + RH \rightarrow ROOH + R\bullet$

Net: $RH + O_2 \rightarrow ROOH$ Eq. 5

Once the initial free radicals are generated, they capture molecular oxygen and form peroxy radicals (ROO•). Then the peroxy radicals in turn can abstract a hydrogen from unsaturated fatty acids to produce a hydroperoxide and the free radical initially generated. This free radical repeats the same reaction just described and the reaction may be repeated up to several thousand times having the nature of a chain reaction. As more hydroperoxides accumulate and decompose to free radicals, this reaction occurs at an accelerated rate. The net reaction of the propagation process (Eq. 5) is the consumption of unsaturated fatty acids with oxygen and the production of hydroperoxides – the primary oxidation products.

For unsaturated fatty acids, the susceptibility to oxidation is dependent on their relative ease to donate a hydrogen for the reaction with peroxy radicals. The free radicals are usually formed at the α positions to double bonds because the bonding energy of the hydrogen atoms at these sites is less and the hydrogen at these sites can be more easily removed by peroxy radicals (Figure 1). The unshared valence electron of the fatty acid free radical formed may delocalize to a resonance structure and be represented by a structure with

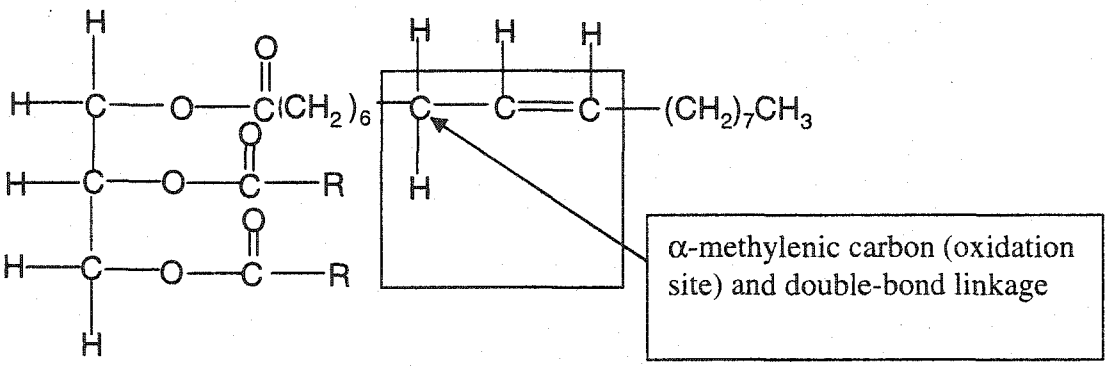


Figure 1. Oxidation sites on unsaturated FA in a triacylglyceride (TAG) molecule. R = FA groups.

a partial free radical at each end of the allylic system (Figure 2). Reaction of oxygen occurs at end carbon positions of the allylic system to produce a mixture of isomeric hydroperoxides (15).

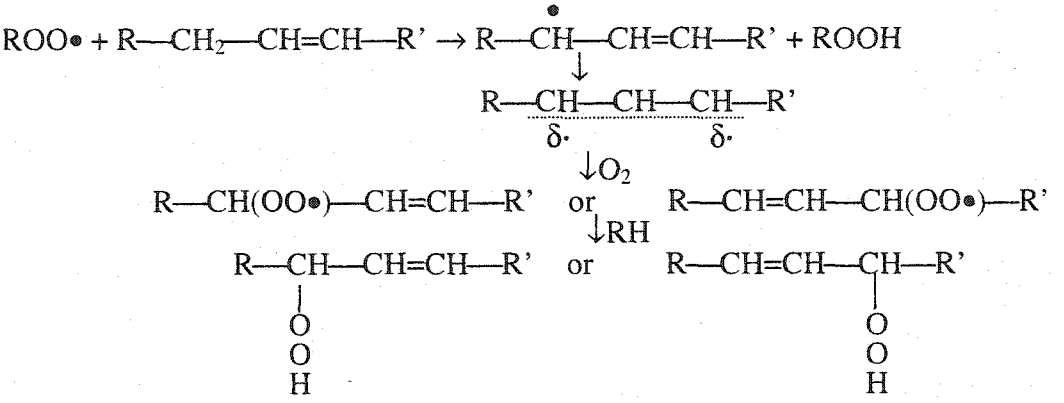
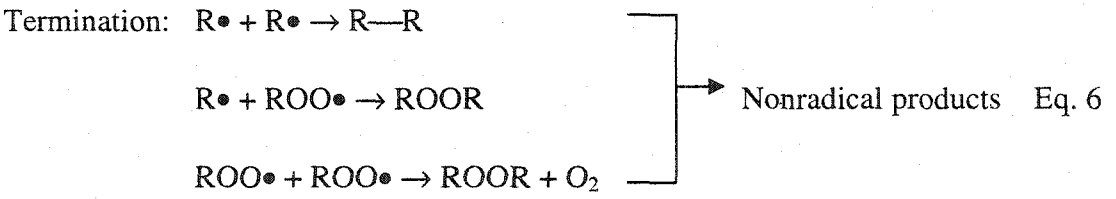


Figure 2. The mechanism of the formation of the mixture of isomeric hydroperoxides.

The propagation can be followed by termination if the free radicals react with themselves to yield nonreactive products.



Photooxidation Another important pathway for the formation of allylic hydroperoxides from unsaturated fats is by exposure to light in the presence of oxygen and a sensitizer. Molecular oxygen in the ground state exists in three closely grouped energy states when placed in a magnetic field. Such a state is called a triplet state and is not very reactive with unsaturated compounds. The activation of triplet oxygen by electronic excitation forms singlet oxygen (single energy state in a magnetic field), which reacts readily with unsaturated

fatty acids. Singlet oxygen can be generated in a great variety of ways as reviewed by Korycka-Dahl (17). The most important way is by exposure to light in the presence of a photosensitizer. Two mechanisms have been postulated for the photooxidation of unsaturated fatty acids (15, 18, 19). In general, olefins undergo photosensitized oxidation by a mechanism in which the sensitizer in the triplet state is excited by visible light energy to the singlet state followed by an intersystem crossing to an activated triplet state (Figure 3, mechanism I). Energy is then transferred from the activated triplet sensitizer to triplet oxygen to give singlet oxygen, which reacts readily with double bonds of unsaturated fatty acids by concerted addition, the so called "ene" reaction. In another postulated mechanism, the triplet sensitizer forms a sensitizer-oxygen complex that reacts with a substrate acceptor (unsaturated fatty acids in this case) to give a peroxide and which regenerates the sensitizer. (Figure 3, mechanism II).

Mechanism I:

$$\text{Sens} \xrightarrow{h\nu} {}^1\text{Sens} \rightsquigarrow {}^3\text{Sens}^*$$

$$^3\text{Sens}^* \xrightarrow{^3O_2} {}^1O_2 + {}^1\text{Sens}$$

$$^1O_2 + \text{RH} \rightarrow \text{ROOH} \quad (\text{RH} = \text{fatty acid acceptor}) \qquad \text{Eq. 7}$$

Mechanism II:

$$\text{Sens} \xrightarrow{h\nu} {}^1\text{Sens} \rightsquigarrow {}^3\text{Sens}^*$$

$$^3\text{Sens}^* + {}^3O_2 \rightarrow {}^1[\text{Sens-}O_2]$$

$$^1[\text{Sens-}O_2] + \text{RH} \rightarrow \text{ROOH} + \text{Sens} \qquad \text{Eq. 8}$$

Figure 3. Mechanisms of photosensitized oxidation (15).

Oxygen is known to be much more soluble in lipids and nonpolar solvents than in water (20), which would provide the source for singlet oxygen formation. Vegetable oils

frequently contain natural photosensitizers, such as chlorophylls and/or pheophytins in refined vegetable oils, are known to be efficient photosensitizers which yield singlet oxygen in the presence of visible light. Singlet oxygen is a highly electrophilic species and reacts readily with moieties containing high densities of electrons, such as the double bonds of unsaturated fatty acids. For example, singlet oxygen reacted with methyl linoleate at a rate of at least 1500 times faster than normal triplet oxygen (20). It was, therefore, concluded that singlet oxygen may play an important role in initiating the free radical autoxidation of unsaturated fats, if one starts with a completely peroxide-free vegetable oil. Once the reaction is initiated by singlet oxygen, the hydroperoxides decompose to yield free radicals, and the mode quickly becomes autocatalytic in the presence of triplet oxygen. A study by Carlsson et al. (21) found that the photooxidation of various unsaturated vegetable oils was not retarded by known free-radical scavengers, but were retarded by compounds known to quench singlet oxygen. Furthermore, the degree of retardation apparently paralleled the singlet oxygen quenching ability of these compounds.

Thermal oxidation Commonly, the fatty acids in food lipids are exposed to heat during processing, and also during cooking, baking, frying, broiling, roasting, canning, concentrating, pasteurizing, drying, etc. Great care should be taken during these processes to minimize thermal oxidation reactions of fats and oils. Thermal reactions are of extreme importance to both consumers and the processors because of their significance to physical and chemical properties and flavor of the foods, nutrition, and toxicity to consumers.

At elevated temperatures, fats and oils can undergo a series of reactions including autooxidative, thermolytic and oxidative polymerization reactions (22). The chemistry of

lipid oxidation is further complicated by the fact that in the presence of air, both thermolytic and oxidative events are superimposed at elevated temperatures.

Not surprisingly, heat treatment such as commercial and household frying, accelerates autoxidation, which has essentially the same pathway as autoxidation at low-temperature, i.e., via the formation and decomposition of hydroperoxide intermediates, which are predictable according to the location and number of the double bonds (22). But at temperatures higher than 80 °C, isolation or quantitation of hydroperoxide intermediates is difficult because they decompose very rapidly. In a study by Lomanno (23), the net peroxide values were 80 and 0 meq/kg, respectively, after heating ethyl linolenate system for only 30 min at 180 and 250 °C, respectively.

In addition to undergoing autoxidation, when fats are heated in the presence of moisture, as often in the case in food applications, fatty acids are released via hydrolysis of the ester linkages, a reaction requiring a molecule of water for each ester group (22). The free fatty acids can accelerate oxidation of the oil. During heat treatment, dimeric and cyclic compounds formation appears to be the predominant thermolytic reaction of unsaturated fatty acids. The mechanism has been explained on the basis of the formation and/or combination of free radicals resulting from homolytic cleavage of C-C linkages near the double bond. Dimeric and cyclic reaction also can occur via Diels-Alder reactions (i.e., reactions between a double bond and a conjugated diene to produce a tetra-substituted cyclohexene). In the presence of oxygen during heat treatment, however, oxidative polymerization also can occur. The alkyl hydroperoxides (ROOH) and dialkyl hydroperoxides (ROOR) formed by autoxidation can readily decompose to form oxy- and peroxy- radicals. Radical combination of such species, addition to double bonds, and allylic hydrogen abstraction leads to the

formation of oxydimers or polymers possessing hydroperoxide, hydroxide, epoxide and carbonyl groups, as well as ether and peroxide bridges (22). Obviously, temperature, heating time and availability of oxygen, etc. can largely influence the extent to which these thermal and oxidative polymerization reactions occur.

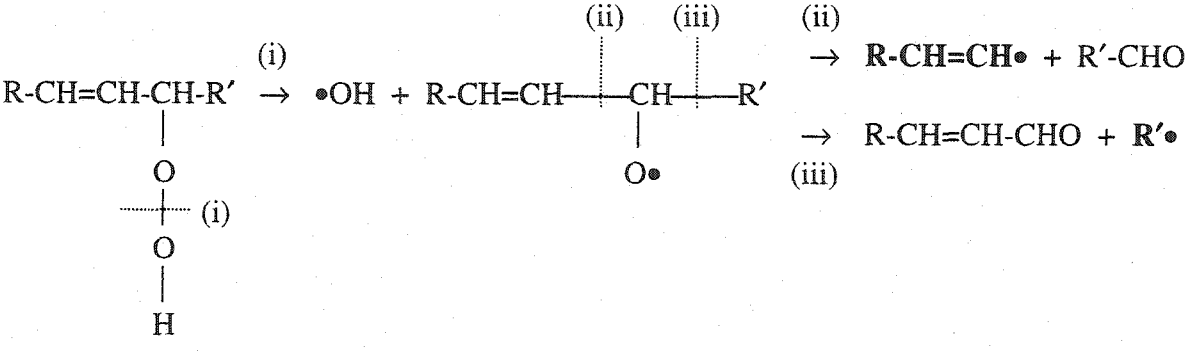
Enzymatic oxidation Enzymes native to plants and animals can initiate oxidation reactions. The most important and best known of these enzymes is lipoxygenase (linoleate:oxygen oxidoreductase, E.C. 1.13.11.12) (LOX), the name of a widely occurring group of enzymes found in most plants and animals (3, 24). Enzymatic oxidations in plant systems are mediated by lipoxygenases that use molecular oxygen to catalyze the oxidation of lipids containing a *cis, cis*-1,4-pentadiene group, such as that present in linoleic and linolenic acid. The reaction leads to the formation of hydroperoxides, same isomers as those formed during autoxidation of linoleate and linolenate. In particular, the activity of three soybean lipoxygenase isozymes, LOX-1, LOX-2, and LOX-3, is greatly associated with the development of off-flavors, especially green-beany flavors, in soybean products (24). In animal systems, lipoxygenases catalyze mainly the oxidative transformation of arachidonic acid to prostaglandins, thromboxanes, and leukotrienes found in all mammalian tissues and having a broad range of biological activities (3).

Lipid Oxidation Products and Their Significances

Primary oxidation products Monohydroperoxides are the primary products of lipid oxidation. A variety of hydroperoxides with positional and geometrical isomers are formed depending on the position and number of double bonds of the unsaturated fatty acids and the

oxidation mechanism. A number of reviews have been published on the composition of isomeric hydroperoxides formed from oxidation of oleate, linoleate, and linolenate (8, 15, 25, 26, 27, 28). From methyl oleate, hydroperoxides with a peroxy group at the positions of 8-, 9-, 10-, and 11- from autoxidation, and at the positions of 9- and 10- from photooxidation were observed. From methyl linoleate, hydroperoxides with a peroxy group at the positions of 9- and 13- from autoxidation, and at the positions of 9-, 10-, 12- and 13- from photooxidation were observed. From methyl linolenate, hydroperoxides with a peroxy group at the positions of 9-, 12-, 13-, and 16- from autoxidation, and at the positions of 9-, 10-, 12-, 13-, 15-, and 16- from photooxidation were observed. The hydroperoxides thus formed are odorless but they are relatively unstable and are the most important precursors of a variety of volatile and nonvolatile secondary products that are important to the flavor stability, physical and chemical properties of SBO in food applications, and to the nutrition and toxicology values for the consumers.

Secondary volatile oxidation products Illustrated by Figure 4 (15, 26, 27) are the pathways of hydroperoxide decomposition and the corresponding products. The first step of hydroperoxide decomposition is the homolytic cleavage of the O-O bond (i) to



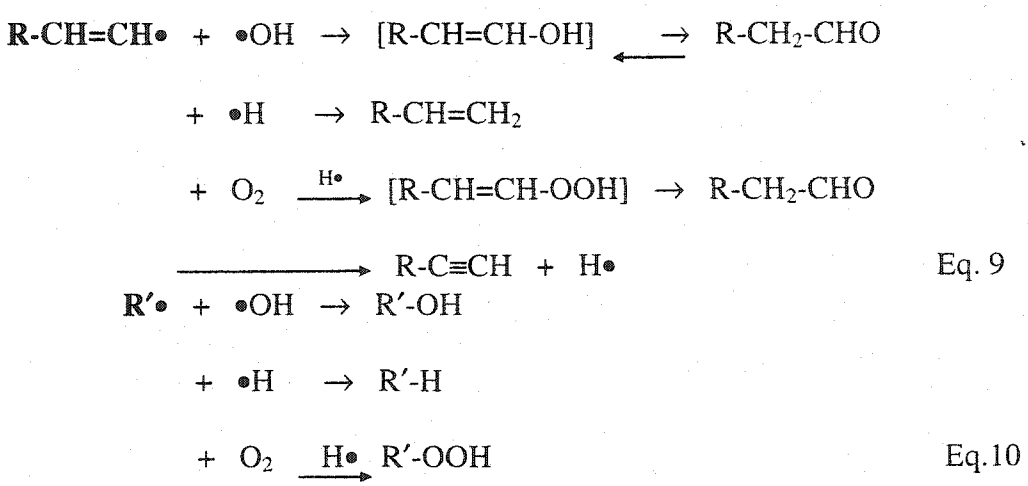


Figure 4. Hydroperoxides decomposition pathways and the secondary volatile products

yield alkoxy and hydroxy radicals. The homolytic β-scission of the C-C bonds (ii) and (iii) of the alkoxy radical leads to two types of aldehydes, an olefin and an alkyl radical, which are the most important free radical reactions leading to breakdown products causing flavor deterioration in fats. The olefin radical formed would be expected to be very reactive and unstable. Further reactions (Eq. 9) may produce aldehydes and alkanes, alkenes, and alkynes. The alkyl radical can undergo similar reactions (Eq. 10) to produce alcohols, hydrocarbons or hydroperoxides. These products can participate in further reactions.

Of these products, volatile products including 2-undecenal, 2-decenal, octanal, nonanal, decanal, heptane, octane, heptanal, 1-heptanol, 1-octanal, 2-nonenal, aldehyde esters, and fatty esters have been identified from decomposition studies with heated methyl oleate hydroperoxides; hexanal, 2,4-decadienal, 2-heptenal, 2-pentylfuran, acetaldehyde, pentanal, 1-pentanol, 1-octen-3-ol, 2-octenal, 2-nonenal, 2,4-nonadienal, esters, a series of C_1 to C_5 hydrocarbons, substituted dioxolanes, ketones, lactones and acids from methyl linoleate hydroperoxides; acrolein, propanal, 2-/3-hexenal, 2,4-heptadienal, 2,4,7-decatrienal, 3-hexen-

1.6-dial, ethane, acetaldehyde, butanal, 2-pentenal, ethyl and 2-butylfuran, 4,5-epoxy-2-heptenal, 3,6-nonadienal, and fatty esters from methyl linolenate hydroperoxides (29).

There is a considerable difference, however, in the flavor significance of these volatile compounds. Frankel (27) reported (Table 1) that hydrocarbons have the highest

Table 1. Flavor Threshold Values of Classes of Volatile Compounds[a]

Class of compound	Threshold value (ppm)
Hydrocarbons	90-2150
Substituted furans	2-27
Vinyl alcohols	0.5-3
1-Alkenes	0.02-9
2-Alkenals	0.04-2.5
Alkanals	0.04-1.0
trans, trans-2,4--Alkadienals	0.04-0.3
Isolated alkadienals	0.002-0.3
Isolated cis-alkenals	0.0003-0.1
trans, cis-2,4-alkadienals	0.002-0.006
Vinyl ketones	0.00002-0.007

[a]Source: Ref. 26.

threshold values and are presumed to have the least impact on flavor. Substituted furans, vinyl alcohols and 1-alkenes also are not particularly significant. In order of increasing flavor significance, vinyl ketones are the most potent with threshold values as low as 0.00002 ppm. Therefore, when estimating the impact of volatile oxidation products on flavor, it is necessary to know not only their relative concentration in a given fat, but also their relative threshold. Table 2 (26) lists volatile carbonyls identified in soybean oil in decreasing order of relative concentration with their corresponding threshold values, in which, *t, t*-2,4-decadienal was the most abundant. If the weighted percentages were calculated on the basis of 1-octen-3-ol, which has the lowest threshold value, the *t, c*-2,4-decadienal becomes the most flavor

important followed by *trans*, *trans*-2,4-decadienal, *trans*, *cis*-2,4- heptadienal, 1-octen-3-ol, n-butanal and n-hexanal.

The impact of these volatile compounds on flavor can be both positive and negative. For example, 3-*cis*- and 3-*trans*-hexenal isolated from reverted soybean oil was

Table 2. Flavor Significance of Soybean Oil Volatiles[a]

Major volatiles	Relative %	Threshold value[b] (ppm)	Weight % (1-Octen-3-ol)	Relative order
t,*t*-2,4-Decadienal[c]	33.7	0.1	2.5	2
t,*c*-2,4-Decadienal	17.9	0.02	6.7	1
t,*c*-2,4-Heptadienal	11.1	0.04	2.1	3
2-Heptenal	5.6	0.2	0.21	8
t,*t*-2,4-Heptadienal	4.5	0.1	0.34	7
n-Hexanal	4.5	0.08	0.42	6
n-Pentane	3.1	340	$6.8 * 10^{-5}$	16
n-Butanal	1.5	0.025	0.45	5
2-Pentenal	1.2	1	0.009	13
1-Octen-3-ol	0.9	0.0075	0.9	4
2- Pentyl furan	0.8	2	0.003	14
n-Pentanal	0.7	0.07	0.075	10
2-Hexenal	0.7	0.6	0.009	13
n-Nonanal	0.7	0.2	0.026	11
n-Heptanal	0.6	0.055	0.082	9
1-Penten-3-ol	0.5	4.2	$8.9 * 10^{-4}$	15
2-Octenal	0.5	0.15	0.025	12

[a] Source: Ref. 30.

[b] Source: Ref. 31.

[c] *t*,*t* = *trans*, *trans*; *t*,*c* = *trans*, *cis*.

described as green-beany (32), but the great amount of γ- and δ- lactones present in coconut oil was thought to contribute positively to its unique flavor and aroma (33). However, it is difficult to agree on common terms for any particular odor or flavor of a fat by sensory panel

and it is controversial about what compounds cause what particular flavors in fats and oils. On the other hand, little progress has yet been made in relating flavor descriptors with individual volatile compounds due to additive and antagonistic interactions between volatile compounds in a natural mixture – food. For instance, Hammond and Hill (34) noted that oct-1-en-3-one accounted for the metallic flavor of autoxidized milk; other researchers identified this compound as a predominant contributor to reverted flavor in soybean oil (35). And you may ask what do you mean by "reverted"?

Crude SBO has a characteristic "green-beany" flavor, which during refining, bleaching and deodorization, is eliminated to produce a bland tasting, light colored oil. However, flavor returns during storage and has been characteristically called the "flavor reversion" of SBO (36). Several theories for the cause of reversion flavor have been proposed (15, 36). Now linolenic acid is widely accepted as the most important precursor of flavor reversion of SBO when oxidized. Efforts, such as reduction of linolenic acid through plant breeding and hydrogenation, have been taken to eliminate "reversion" flavor of SBO. The term "reversion" is a misnomer since the flavor formed upon aging is not exactly the same as the raw "green beany" flavor typical of crude oil before processing into finished oil (36).

Secondary nonvolatile oxidation products Decomposition and condensation of hydroperoxides produces a multitude of nonvolatile monomeric products, including di- and tri-oxygenated esters, dimeric and polymeric materials, especially at elevated temperature. Many of these dimers and polymers are known to be rich sources of volatile carbonyl compounds and to decrease the flavor and oxidative stability of SBO (37). These high-

molecular-weight materials also can produce a series of physical and chemical changes to the oil and food products, including increased viscosity, polarity, free acid content, development of dark color, and an increased tendency of the oil to foam (22).

Factors Affecting Fat and Oil Stability and Quality

Fatty acid composition Fatty acids differ in their susceptibility to oxidation; thus, fatty acid make-up of an oil has a major effect on its stability and flavor quality. Fatemi et al. (7) measured the relative rates of oxidation of the pure oleate, linoleate, and linolenate fatty esters as 1:10.3:21.6. However, it is difficult to predict the contribution of different fatty acids in promoting oxidation when present in mixtures as is the case in natural fats. Some studies showed significant interactions between different unsaturated fatty esters (38, 39). With equal mixtures of oleate, linoleate and linolenate, the respective ratio of hydroperoxides corresponding to the specific fatty acid was 1:4.3:5.8 at a peroxide value of 114 and 1:6.3:3.7 at a peroxide value of 563. Therefore, at the more advanced level of autoxidation, the proportion of linolenate hydroperoxides detected was less than that of linoleate and greater than that of oleate hydroperoxides. Based on the susceptibility of fatty acids to oxidation, removal of fatty acids that oxidize quickly might be used to improve stability and quality of SBO. Therefore, reduction of linolenic acid and elevation of oleic acid through plant breeding can be used to achieve this goal.

Triglyceride structure Some researchers (40) have observed that normal soybean oil randomly interesterified with stearate was far less stable than when stearate was placed selectively on the *sn*-1 and *sn*-3 positions. Although the reasons for the effect are not

fully understood, most experts now agree that the placement of fatty acids within the triacylglycerol does have an effect on oxidation. The implication to the fats and oils industry is that it is possible to alter (increase or decrease) the oxidative stability of a native oil by randomization (8).

Free fatty acids, mono- and diglycerides, and phospholipids Fatty acids may be cleaved from the glycerol backbone by action of enzymes native to the plant or animal from which the oil is extracted. Free fatty acids oxidize slightly more quickly than when esterified to the glycerol backbone; they can catalyze the oxidation of the entire bulk of the oil; Catalytic trace metals from oil processing and storage equipment can attach to the free fatty acids and thus accelerate oxidation of the oil (8). The presence of mono- and/or diglycerides also reduces the oxidative stability of an oil (41). Phospholipids, present in crude soybean oil at ~1.5%, have been reported as anti- and prooxidants, depending on a number of other factors (42). Fortunately, these components are nearly completely removed from vegetable oils during refining, bleaching, and deodorization to produce a stable product.

Native antioxidants The stability of many vegetable oils has been credited to the presence of the native tocols and other natural antioxidants (8). Tocols include four tocopherol and four tocotrienol isomers, each designated as α, β, γ, or δ on the basis of methylation of the chromanol ring. They are one kind of phenolic compound that is widely distributed in plants, and are important to controlling oxidative processes in both plants and the extracted oils. They inhibit lipid oxidation in foods and biological systems by stabilizing hydroperoxy and other free radicals (43). Lard, long considered to be oxidatively unstable

because of its lack of natural antioxidants, especially tocopherols, would benefit from having a greater concentration of native antioxidants as shown by Marinova et al. (44). The α-tocopherol also acts as a singlet oxygen quencher preventing photooxidation of fats and oils. Other native antioxidants, including flavonoids, phenols, phenolic acids and their derivatives, terpenoids such as carsonic acid, canosol, rosmarinic acid, rosmaridiphenol and rosmanol also demonstrate antioxidant acitivity as free radical acceptors and as chain breakers in different food systems (45).

Other minor constituents frequently found in fats and oils, such as the two fat-soluble pigments, chlorophyll and carotenoids, may act as photosensitizers and singlet oxygen quenchers in the light. The presence of chlorophyll in canola and soybean oils, a common problem in immature seed, is generally agreed to reduce oxidative stability during storage. The carotenoids, β-carotene and lycopene, are particularly effective at quenching singlet oxygen, especially at the low oxygen pressures. Growing evidence also indicates that a significant amount of photosensitizers is still left in bleached-deodorized SBO to contribute to its light instability (46, 47) and refining and bleaching also remove singlet oxygen quenchers, such as the carotenoids.

Some plant sterols, including Δ^5–avenasterol, Δ^7–avenasterol, fucosterol, citrostadienol, vernosterol, isolated from the unsaponifiable fraction of olive, corn, wheat, and *Vernonia anthelmintica* oils have shown anti-polymerization activity in heated oils (48).

External factors: light, oxygen, temperature, surface area, water activity, metals and added antioxidants In addition to factors inherent in the composition of an oil, any external factors that contribute to lipid oxidation reactions can affect oil stability and quality.

The presence of light and oxygen promotes lipid oxidation (49). The rate of reaction is greater at high temperatures than at lower temperatures and oxidation increases with an increase in the surface area of fat or oil that is exposed to air (49). For a pure edible oil, the oxidative stability is generally greatest at extremely low water activity, where hydrolysis of the fatty acids from the glycerol backbone is unlikely (8). Transition metals, particularly those with two or more valence states, are prooxidants. They can come from metallic equipment used in oil processing or storage or from the soil in which an oil-bearing plant was grown. Thus, it is extremely difficult to remove trace metals completely from fats and oils. For this reason, metal chelators, especially citric acid, are typically added to fats and oils during processing (8). Synthetic antioxidants, such as monotertiary butylhydroquinone (TBHQ), butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT), and propyl gallate (PG), in addition to those naturally present in oils, are typically added to fats and oils to reduce and slow the rate of oxidation. The chemical compound, polydimethylsiloxane, is also widely applied in aqueous systems and in frying to suppress foaming and polymerization (50).

Measures to Improve Fat and Oil Stability and Quality

Hydrogenation Hydrogenation is an important process for maintaining flavor stability and is the basis for the shortening, margarine and salad oil industries (15). During hydrogenation, gaseous hydrogen, liquid oil, and a solid catalyst, such as nickel or palladium, interact under agitation in a closed vessel. Generally, hydrogenation of fats is not carried to completion, and fats are just partially hydrogenated providing only a partial solution to improving flavor stability of SBO. Under these conditions, hydrogenation may be selective

or nonselective. "Selective" means that hydrogen is added first to the most unsaturated fatty acids. The selectively hydrogenated oil is more resistant to oxidation because of the preferential hydrogenation of the linolenic acid. Another important aspect of hydrogenation is the formation of *trans* fatty acid isomers due to the reversible character of chemisorption (3, 51).

Plant breeding Another method to improve stability and flavor quality of SBO through altering fatty acid composition is via plant breeding. The tools used by plant breeders have been selections, crossing, mutation and genetic engineering (10). By analyzing a large number of seeds for their fatty acid composition, seeds with desired fatty acid composition can be selected for future crop development. Further by crossing plants with special fatty acid composition with plants of normal fatty acid composition, offspring seeds with desired fatty acid composition can be developed. Mutation involves treating seeds or plants with mutagenic materials, such as gamma rays or sodium azide and then analyzing the offspring seeds from the treated parents for fatty acid composition, to find seeds with desirable modifications (52). Genetic engineering, including such techniques as recombinant DNA, gene transfer, tissue culture and plant regeneration, involve direct gene manipulation and can help to reach goals that are difficult to achieve by conventional breeding. Plants bred through genetic engineering, however, must deal with international regulatory issues and consumer resistance to these genetically modified organism (GMO) crops. Also, although quality enhancement of vegetable oils can be achieved through both plant breeding and hydrogenation, the former has become increasing popular because it produces *trans* free oils, whereas commercial hydrogenation creates oils with *trans* double bonds. Low-linolenic,

high-oleic, low-saturated and high-saturated vegetable oils through plant breeding have become available for targeted applications (9).

Antioxidants Autoxidation can be inhibited or retarded by adding low concentrations chain-breaking antioxidants (AH) that interfere with either chain propagation or initiation (15). Chain-breaking antioxidants include phenolic and aromatic compounds hindered

$$ROO\bullet + AH \rightarrow ROOH + A\bullet \qquad \text{Eq. 11}$$

with bulky alkyl substituents. Common synthetic chain-breaking antioxidants used in food lipids include BHA, BHT, TBHQ, and PG. The antioxidant radical (A•) formed in Eq. 11 should be stable and unable to initiate or propagate the oxidation chain reaction. The phenolic antioxidants achieve stability by forming resonance hydrids (Figure 5.) (50). A radical intermediate, such as, semiquinone, can further undergo a variety of reactions including dismutation to form a stable quinone and can regenerate the original hydroquinone (Figure 6) (50). However, these antioxidants generally lose their efficiency at elevated temperatures and they are most effective during the induction period. Once the antioxidant is consumed, oxidation accelerates (50).

Preventive antioxidants reduce the rate of the chain initiation. The most important initiation suppressors are metal deactivators that chelate metal ions that catalyze chain initiation. Metal deactivators used for stabilizing edible fat and lipid-containing foods include citric, phosphoric, tartaric acid, and phospholipids. Peroxide destroyers also are preventive antioxidants; for example, sulfur compounds, phosphates and phosphines reduce hydroperoxides into more stable alcohols (15). Ultraviolet light deactivators can prevent

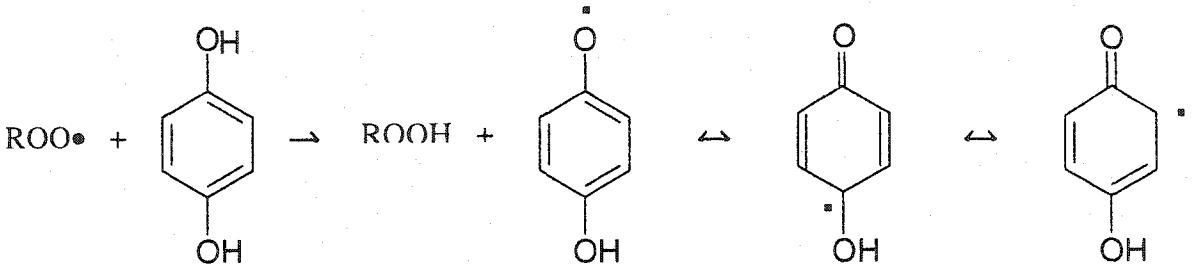


Figure 5. The formation of resonance hydrids by the phenolic antioxidants

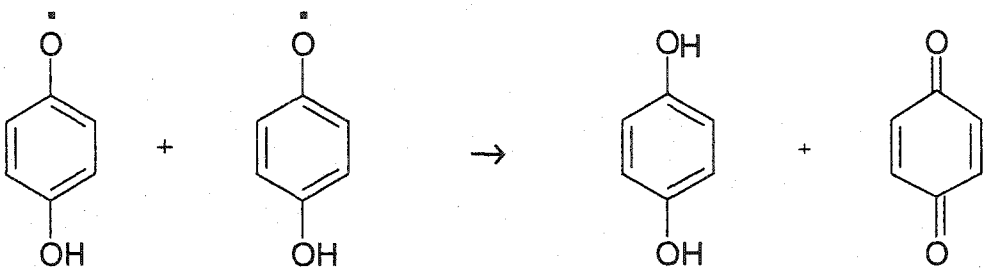


Figure 6. The dismutation of a semiquinone radical intermediate

oxidation by absorbing irradiation without the formation of radicals. Examples include pigments such as carbon black, phenyl salicylate, and α-hydroxy-benzophenone. A significant synergistic antioxidative effect can be achieved when chain-breaking and preventive antioxidants are used together, because they suppress both initiation and propagation. The synergistic effect of common antioxidants in combination with metal inactivators in foods has been known for a long time (53). Loliger (45) showed that the tertiary antioxidant system of vitamin E, vitamin C, and phospholipid provided the best

protection against oxidative degradation among when compared to the two antioxidants used alone or in combination.

Processing and storage with minimum exposure to oxidation Good processing and storage measures include careful control of refining temperature, vacuum bleaching, inert gas blanketing, low temperature and protection from light during storage. Vacuum conditions are very important during bleaching, because oxidation can readily occur by exposure of a large surface area to air at elevated temperatures. Refining and bleaching remove not only natural photosensitizers but also singlet oxygen, thus they may upset the natural balance between de-stabilizing photosensitizers and stabilizing quenchers, such as carotenoids. The restoration of carotenoids may effectively protect lipids against singlet oxygen deterioration, but the resulting yellow coloration maybe objectionable to the consumer. Another approach to protecting stored oils is the use of a package or container that is absorbent to the light energy necessary for photosensitization, or that prevents such light from reaching the oil. Also, displacement of oxygen in a container by nitrogen or carbon dioxide to $\leq 2\%$ has been shown to reduce oxidation effectively in vegetable oil (54).

Methods to Measure Stability and Quality of Fats and Oils

Peroxide value (PV) The PV, expressed as milliequivalents of peroxide per kilogram of oil (mEq/kg), measures the primary oxidation products of oils – hydroperoxides. Assessment of the PV of an oil during storage is quite common, and fairly useful. It is said to be an index to the oxidative state of an oil. For SBO, an oil is considered to be "fresh" with PV of <1.0, to have low oxidation with a PV of 1.0-5.0, to have moderate oxidation at a PV

of 5.0-10.0, to have high oxidation at a PV >10.0, and to have poor flavor quality at a PV >20 mEq/kg oil. Several methods (55, 56, 57, 58) can be used to measure PV of an oil depending on the specific circumstance.

Conjugated diene value (CD) One of the first steps in the oxidation of PUFA in an oil is a shift in the position of the double bonds and resulting in the formation of conjugated hydroperoxides. The conjugated structure absorbs strongly at a wavelength of 232-234 nm. The CD value by this method (56) is expressed as percentage of conjugated dienoic acid in the oil and is an indication of initial or primary oxidation products. The CD can be used as a comparative method only when the oils have the same initial FA composition, because the greater the amount of PUFA in an oil, the greater the potential rise in CD. Therefore, it should be used as a relative measurement of oxidation in an oil only if the fatty acid composition is known (58).

p-Anisidine value (p-AV) The method (56) measures light absorbance of aldehydes at 350 nm, primarily 2-alkenals, and 2,4-dienals. But it is not entirely specific because the color intensity developed depends not only on the concentration but also on the actual structure of the aldehyde. Therefore, the result is comparable only within an oil type because of the initial difference in the value among oil sources (5 9).

Free fatty acid (60), Polar compound (56), Viscosity, and Color These chemical analyses are often performed to determine the degree of abuse of oils during heating or frying. They are important indicators for frying oil administration and also have effect on the

quality of the fried food. The FFA increases during frying indicating increased fatty acids are released from TAG ester linkages via hydrolysis (22). Thus, it is an important marker for oil quality. Extremely abused frying oil should be discarded based on a German standard of 27% total polar compound as an indicator of poor frying oil quality (61). Changes of viscosity and color of the frying oil are also used as indicators of extent of frying oil degradation.

Other chemical methods of analysis There are many other methods for measuring lipid oxidation and quality by chemical means. A few of the best-known procedures include thiobarbituric acid test, carbonyl value, and headspace oxygen analysis. These methods are reviewed and discussed by other researchers (8, 62).

Volatile compound analysis by gas chromatography (GC) The volatile carbonyl compounds from oxidation in fats and oils are major contributors to off-flavor development as discussed previously. Therefore, there has been significant effort at identification and quantification of these compounds. It is difficult to analyze these compounds in fats and oils because of several reasons. It is difficult to remove them from the fats and oils; widespread contamination by carbonyls in the experiment solvents, glassware, and other materials used in the laboratory may cause artifacts to the results; and hundreds of volatile compounds may be formed in fats and oils during oxidation causing difficulties in the interpretation. Not until the recent use of efficient GC columns and proper means of identification has the volatile compound analysis become possible.

Three basic GC procedures are generally employed (56), including static headspace, dynamic headspace, and direct injection. Static headspace involves equilibration of gases

from the area above a liquid sample; a set volume of the headspace gas from the sample is then injected directly into the GC for separation and quantification. The dynamic headspace method, also known as purge and trap, employs a sorbent, such as Tenax GC, Chromosorb, or Porapak Q., to collect volatile compounds which are swept from a heated sample with nitrogen. After trapping, the sorbent may be extracted with solvent, or transferred directly to the GC. In direct injection, an oil sample may be injected directly into the port of GC through a silanized glass wool plug. Each of these methods has their own advantages and disadvantages (8).

Recently, the method of GC Solid-Phase Microextraction (GC-SPME) has been developed (63, 64, 65). It uses a fiber coated with different polymers to extract volatile compounds from a food system. The method can be used in solid, liquid, and gaseous systems. It is not difficult to do the analysis at a consistent condition. The results obtained in our laboratory and by other researchers are very good. More details of the procedure can be found in the materials and methods section of the fourth paper in this dissertation.

Sensory evaluation The ultimate method to assess oil quality and stability is sensory analysis, which can not be replaced by any chemical or instrumental analysis, although some methods can correlate fairly well with this overall evaluation. Sensory evaluation of oils should be done by a panel of experts or a trained panel according to the method described by the American Oil Chemists' Society (56). In actual evaluation, usually, the panel is asked to score the overall flavor quality, and as well as the intensity of many individual off-flavors. The number of flavors that can be present in soybean oil can be as many as 15, or more (56). Therefore, the resulting data are multivariate, because they are

made up of complex interrelated elements. The standard display of data, such as numbers, may obscure the recognition of relationships among elements. To make overall perception and interrelationships immediately apparent, and to provide a more accurate judgment as a well-integrated pictorial display, the second paper of this dissertation is an attempt to apply one of the multivariate data presentation methods in sensory evaluation of vegetable oils.

Oxidative and Flavor Stabilities of Soybean Oils with Low and Ultra-Low Linolenic Acid Composition

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Caiping Su[a], Monoj Gupta[b], and Pamela White[a,]*

[a]Department of Food Science and Human Nutrition and Center for Crops Utilization Research, Iowa State University, Ames, Iowa 50011

[b]MG Edible Oil Consulting Intl, Richardson, Texas 75080

Running Title: OXIDATIVE AND FLAVOR STABILITIES OF SOYBEAN OILS

* To whom correspondence should be addressed at 2312 Food Sciences Building, Iowa State University, Ames, IA 50011-1060. Phone: 515-294-9688, Fax: 515-294-8181, Email: pjwhite@iastate.edu

ABSTRACT: The effects of linolenic acid (18:3) concentration, combined with TBHQ addition, temperature, and storage time, on the oxidative and flavor stabilities of soybean oils (SBO) were evaluated. During storage under fluorescent light at both 21°C and 32°C, the SBO with ultra-low-18:3 concentration (1.0%, ULSBO) generally had greater oxidative stability than did SBO with low-18:3 concentration (2.2%, LLSBO). The ULSBO had about half the p-anisidine value of LLSBO throughout the storage. Although the ULSBO initially had significantly greater peroxide values and poorer (lower) sensory scores for overall flavor

quality than did LLSBO, significant differences disappeared with storage. The ULSBO had a lower content of polar compounds and greater oil stability indices than did LLSBO when TBHQ was present. All oils were more oxidatively stable with TBHQ addition, but the TBHQ addition did not result in improved flavor stability early in storage. In all tests, oils stored at 32°C were less stable than oils stored at 21°C. The TBHQ had a better antioxidant capacity when the 18:3 concentration was lower. The retardation effect of TBHQ on lipid oxidation and the improved stability of ULSBO over LLSBO were more easily detected when the storage temperature was higher.

KEY WORDS: Fatty acid composition, flavor stability, linolenic acid concentration, oxidative stability, *soybean oil.*

Soybean oil (SBO) has a good nutritional profile because of its high proportion of unsaturated fatty acids, but SBO has poor oxidative stability and is prone to flavor deterioration. The fatty acid, linolenic acid (18:3), oxidizes very quickly and is the most important precursor of flavor deterioration in 18:3-containing oils (1, 2). Hydroperoxides formed by oxidation of 18:3 can break down to many undesirable flavor compounds such as 2,4-heptadienal, 2-butylfuran, 2- and/or 3-hexenal, 2-pentenal and butanal (3). To improve oxidative stability and flavor quality, the SBO may be hydrogenated to reduce the concentration of polyunsaturated fatty acids; however, *trans* fatty acids (*t*FA) are formed during this process. Because of health concerns over the presence of *t*FA in our diets (4, 5), lowering the 18:3 content to a level similar to that obtained by partial hydrogenation, but without *trans* formation, has been an objective of plant breeders. Another advantage to

producing oils needing no additional processing is that fewer processing costs should result in more profit for farmers and processors (6). Previous studies (7, 8, 9) determined that the oxidative and flavor stability of oils were inversely proportional to the initial 18:3 concentration. Although considerable information is available regarding the relationship between oxidative and flavor stability of SBO and 18:3 concentration, soybean breeders need more precise compositional targets to produce SBO that have good oxidative and flavor stability. The objective of this research was to study the effects of two low levels of 18:3 concentration (~1.0% and 2.2%) combined with TBHQ addition, temperature, and storage time on the oxidative and flavor stabilities of SBO.

MATERIALS AND METHODS

Soybean oils and design. Soybeans (*Glycine max*) with low-18:3 (2.2%) and ultra-low-18:3 (1.0%) concentrations, grown in summer 2000 in Iowa (weather zone 2), were obtained from Protein Technologies, Inc. (St. Louis, MO). The LL soybeans were crushed in Montolla, MN, and the UL soybeans were crushed at the POS Pilot Plant Corporation in Saskatoon, Saskatchewan, Canada. Both oils were hexane-extracted, and refined, bleached, deodorized, and bottled at the POS Plant. Citric acid (50 ppm) was added to the oils during the cool-down stage of deodorization. The antioxidant, TBHQ (100 ppm), was added to half of each oil type at the deodorization step before bottling in co-extruded polyethylene terephthalate (PET) plastic bottles. The bottles were sparged with nitrogen until they contained less than 2% oxygen in the headspace, then sealed. Bottled oils were sent to Iowa State University (ISU) (Ames, IA) for evaluation. Thus, four SBO treatments were tested, including low-18:3 SBO (LLSBO), LLSBO with the addition of 100 ppm TBHQ (LLSBOW), ultra-low-18:3 SBO

(ULSBO), and ULSBO with the addition of 100 ppm TBHQ (ULSBOW). For each of these four treatments, two bottles were retained at arrival, and the remaining bottles were stored under fluorescent light with uniform exposure of 70-foot candle light intensity at 21°C and 32°C, respectively, for 12 months. Duplicate bottles of oil from each treatment were analyzed in duplicate at 0, 2, 4, 6, 8, 10, and 12 months of storage.

Chemicals. Tetrachloroethane (98+%), lauroyl peroxide (97%), p-anisidine (99%), and sodium methoxide (0.5 M solution in methanol, A.C.S. reagent) were purchased from Aldrich Chemical Co. (Milwaukee, WI). Iso-octane, s-diphenylcarbazide, ethyl ether, acetic acid glacial (certified A.C.S. grade), and petroleum ether (Optima) were purchased from Fisher Scientific Inc. (Fair Lawn, NJ). Silica Gel 60, particle size 0.063–0.200 mm, was from E. Merck Science (Gibbstown, NJ). The individual tocopherols, including *d*-α-tocopherol, *d*-γ-tocopherol, and *d*-δ-tocopherol, (90% pure) were purchased from Sigma-Aldrich, Inc. (St. Louis, MO).

Fatty acid composition by GC. Fatty acid compositions of SBO were determined by converting TAG into FAME according to a method described by Hammond (10). The GC conditions were the same as described by Shen *et al.* (6).

Tocopherol contents by HPLC. Tocopherol contents of the oils were determined according to AOCS Official Method Ce 8-89 (11) by using the System Gold® HPLC equipped with a UV detector and solvent miser silica 5u column (length 250 nm, ID 2 mm; Alltech Associates, Inc., Deerfield, IL). Tocopherol content in native soybean seeds was obtained from oil extracted with hexane after crushing the seed with a hydraulic press, as described by Hammond (10).

Oil stability indices (OSI). The OSI were analyzed according to AOCS Official Method Cd 12b-92 (11) with the Oxidative Stability Instrument (Onion, Inc., Rockland, MA) at 110°C with an air flow rate of 150 mL/min.

Peroxide values (PV). The PV was determined by the Stamm test as modified by Hamm *et al.* (12). The commercially available tetrachloroethane was purified by the following steps: adding 1% lauroyl peroxide, heating in a boiling water bath for 1 h, distilling at 60°C by using a rotary evaporator, adding 0.2% s-diphenyl carbazide, heating in a boiling water bath for 1 h, distilling at 60°C with the rotary evaporator and, finally, collecting the purified solvent from the receiver flask. Purity of the solvent was judged by having a nil or nearly nil reading at 565 nm on a spectrophotometer.

p-Anisidine value (p-AV). The p-AV was measured by using AOCS Official Method Cd 18-90 (11).

Polar compounds. The percentage of polar compounds was measured according to AOCS Official Method Cd 20-91 (11).

Lovibond colors (Colors). Colors were measured based on AOCS Official Method Cc 13e-92 (11) by using an AOCS Tintometer AF710 with a sample tube depth of 5 ¼ ″ (13.3 cm).

Sensory evaluations. Sensory evaluations were conducted according to AOCS Recommended Practice Cg 2-83 (11). A 15-member trained descriptive panel was used to evaluate overall flavor quality and individual off-flavor intensities of SBO. All panelist candidates were trained during three 1.5-h sessions. During training, panelists were given standards for off-flavor characteristics found in SBO. These standards included fresh SBO purchased from a local store, and SBO treated to have buttery, grassy, and painty flavors, and

a bitter taste (0.1% caffeine in commercial fresh SBO), respectively, prepared according to the AOCS method Cg 2-83 (11). Panelists who could not recognize these standards after training were omitted as panelists.

For the actual tests, the SBO were held at 50°C; placed in plastic cups labeled with random, three-digit codes; and presented in random order to panelists. To avoid tasting fatigue and flavor carry-over, panelists were asked to expectorate the sample after tasting and to rinse their mouths with distilled water between tasting samples. Tests were conducted in individual, lighted booths. The oils were evaluated for overall flavor quality on a 10-point scale (10=excellent quality, 9 and 8=good, 7 and 6=fair, 5 and 4=poor, 3, 2, and 1=very poor) and for intensity of individual flavors described by the AOCS method Cg 2-83 (11) on a 10-point scale (10=bland, 9=trace, 8=faint, 7=slight, 6=mild, 5=moderate, 4=definite, 3=strong, 2=very strong, 1=extreme). Individual flavors included nutty, buttery, corny, beany, hydrogenated, burned, weedy, grassy, rubbery, melon, painty, and fishy. Overall flavor quality scores were calculated as the average of all scores given by the panelists. Intensity of a flavor was calculated as the average of the intensity scores by the panelists who detected the flavor.

Triangle tests were done following standard procedures (13) to determine whether the overall flavor characteristics between SBO, with and without TBHQ addition, were different.

Statistical analysis. Data were analyzed as a randomized $2 \times 2 \times 2 \times 7$ factorial experiment. Data from all treatments were analyzed by general linear models procedure (program GLM) (14). Differences in mean values among treatments were determined by the least significant difference test at $\alpha = 0.05$, unless listed otherwise.

RESULTS AND DISCUSSION

Fatty acid composition, calculated oxidizability, iodine value (IV) and *Totox value*. Initially, all the ultra-low-18:3 SBO treatments contained similar amounts of 16:0 and 18:0, slightly more 18:1, slightly less 18:2 and less 18:3 (1.0%), than did all the low-18:3 SBO treatments (2.2% 18:3) (Table 1). Values for calculated oxidizability and IV suggest that all the ultra-low-18:3 SBO treatments would be more stable than all the low-18:3 SBO treatments. There were no differences in fatty acid composition, calculated oxidizability, or IV between LLSBO and LLSBOW and between ULSBO and ULSBOW. The fatty acid composition of all oils did not change during storage at 21°C or 32°C for 12 months.

Tocopherols. Initially and after 12-month storage, the ULSBO and ULSBOW contained much less α-, γ-, δ-, and total tocopherols than did LLSBO and LLSBOW (Table 2). The ULSBO and ULSBOW had less total loss and slightly less % of total loss than did LLSBO and LLSBOW, suggesting that tocopherols in ULSBO and ULSBOW were less consumed or exhausted than in LLSBO and LLSBOW.

To determine whether the differences in tocopherol contents between the ultra-low and low-18:3 SBO were inherent in the beans or resulted during processing, seeds from two lines of UL and three lines of LL soybeans grown in four different environments, and of same genetic background as those used in the current study, were analyzed (Kristen McCord, personal communication). There were no differences in the concentrations of tocopherol homologues or total tocopherol concentration between the UL and LL SBO, or among the different growing environments. A tendency observed by Shmulovich (15) for increased polyunsaturation of soybean oil with increased tocopherol content did not exist in the current study. Thus, the differences in the tocopherol concentrations found in the processed oils used

in the current study were likely a result of processing. None-the-less, and despite the lower tocopherol levels, ULSBO showed better stability than did LLSBO as discussed in the following sections.

Oxidative stability indices. The OSI of all SBO treatments decreased during storage, suggesting a decrease in oxidative stability overall (Table 3). Throughout storage, oils with TBHQ addition had significantly greater OSI than did the oils without TBHQ addition for the same 18:3 concentration and storage temperature. The LLSBO tended to have greater OSI values than did the ULSBO when TBHQ was absent and at the same storage temperature, but differences were small and not usually statistically significant. When TBHQ was present, the opposite trend was observed; that is, the ULSBOW had greater OSI than did LLSBOW at the same storage temperature. The statistical analysis for a null interaction hypothesis between the effects of 18:3 content and TBHQ addition on OSI revealed an interaction ($p < 0.001$). Oils stored at 21°C had greater OSI than did the oils stored at 32°C with the same 18:3 content and TBHQ level. But, in general, the differences were significant only when TBHQ was present, which suggests an interaction between the effects of temperature and TBHQ addition on OSI. Statistical analysis demonstrated an interaction ($p = 0.0061$) between the effects of temperature and TBHQ addition on OSI. The antioxidant, TBHQ, is a common chain-breaking antioxidant used in food lipids to interfere with either chain propagation or initiation of lipid oxidation via free radical reactions (2).

These results and interactions between the effects of 18:3 content and TBHQ addition, and between the effects of temperature and TBHQ addition on OSI, showed that TBHQ had a better antioxidant capacity when the 18:3 concentration was lower. The retardation effect of TBHQ on lipid oxidation was detected more easily when the storage temperature was higher.

Peroxide values. The effects of the treatment factors (18:3 concentration, TBHQ addition, and storage temperature) on PV were complex. Statistical analyses of the data showed interactions between the effects of 18:3 concentration and temperature ($p = 0.0006$); between the effects of 18:3 content and TBHQ addition ($p < 0.0001$); and among the effects of 18:3 content, TBHQ addition, and temperature ($p = 0.0625$, close but not statistically significant) on PV.

When TBHQ was absent and at the same storage temperature, the ULSBO initially had significantly greater PV than did LLSBO (Table 3). But the trend reversed during storage by 10 months at 21°C and by 8 months at 32°C. The interaction between the effects of 18:3 concentration and temperature on the PV suggests that the improved stability of ULSBO over LLSBO appeared sooner at a higher storage temperature. When TBHQ was present, at 21°C, the ULSBOW had higher PV than did the LLSBOW; at 32°C, the ULSBOW had lower PV than did LLSBOW. The interactions between the effects of 18:3 content and TBHQ addition and among the effects of 18:3 content, TBHQ addition, and temperature on PV suggest that TBHQ had a better antioxidant capacity when the 18:3 concentration was lower. The retardation effect of TBHQ on lipid oxidation and the improved stability of ULSBO over LLSBO were more easily detected when the storage temperature was higher.

The TBHQ addition had a great effect on PV (Table 3). As storage progressed, all the oils with TBHQ addition had lower PV than did the oils without TBHQ addition for the same 18:3 concentration and storage temperature. Also, temperature played an important role in the formation of lipid hydroperoxides. During storage, oils stored at 21°C generally

developed lower PV than did oils stored at 32°C for the same 18:3 concentration and TBHQ level, although the differences were not always significant.

p-AV. Throughout storage, ULSBO had significantly lower p-AV than did LLSBO at the same temperature and TBHQ levels, except for oils with TBHQ stored at 32 °C for 8 months (Table 3). Such results are in agreement with descriptions by other researchers who noted differences in p-AV of oils with different fatty acid compositions (11, 16). After storage began, oils with TBHQ addition had lower p-AV than did oils without TBHQ addition at the same 18:3 concentration and storage temperature except for LLSBO at 21°C and at 2- and 10-month storage. This result and the interactions between the effects of 18:3 concentration and TBHQ addition ($p = 0.0011$), storage temperature and TBHQ addition ($p = 0.0016$), and 18:3 concentration and storage temperature ($p < 0.0001$) on p-AV again suggest that TBHQ had a better antioxidant capacity when the 18:3 concentration was lower. The retardation effect of TBHQ on lipid oxidation and the improved stability of ULSBO over LLSBO were more easily detected when the storage temperature was higher. After two months, oils stored at 32°C had significantly greater p-AV than did oils stored at 21°C with the same 18:3 concentration and TBHQ levels, except for LLSBO with TBHQ at 8-month storage (Table 3).

The p-AV method determines the amount of aldehydes (principally 2-alkenals and 2,4-dienals) present; however, the color intensity of the yellowish reaction products formed depends not only on the amounts of aldehydic compounds present but also on their structure (11). A double bond in the carbon chain conjugated with the carbonyl double bond increases the molar absorbance by four to five times, that is, the 2-alkenals and dienals, especially, contribute substantially to the value found. Oils with high PUFA levels may have p-AV of

greater than 10.0 mmol/kg even when fresh, largely because of the structure of the aldehydes (17). The p-AV is comparable only within an oil type because of the initial difference in the value (16).

The Totox value, taking into account the limit of the p-AV method, was calculated as the sum of p-AV and 2PV as shown in Table 1 (16). Initially, ULSBO had lower Totox than did LLSBO. There were no differences in Totox between LLSBO and LLSBOW or between ULSBO and ULSBOW. By the end of 12-month storage, ULSBO still had lower Totox than did LLSBO (Table 1).

Polar compounds. Generally, ULSBO had lower polar compound percentages than did LLSBO at the same temperature and TBHQ level, especially as storage progressed (Table 3). At 32°C, oils with TBHQ addition tended to have lower values than did the oils without TBHQ addition at the same 18:3 level, especially as storage progressed. There was no such trend at 21°C. Statistical analysis confirmed the interaction between the effects of temperature and TBHQ addition ($p < 0.0001$) on polar compound percentages. Oils stored at 21°C had lower values than did the oils stored at 32°C when TBHQ was absent, especially as storage progressed. These results and the interaction again suggest that the retardation effect of TBHQ on lipid oxidation was more easily detected when the storage temperature was higher.

Colors. There were no interactions between the effects of 18:3 concentration, temperature, or TBHQ addition on color changes. Initially, ULSBO (3 yellow, 0.2 red) and ULSBOW (3 yellow, 0.2 red) had significantly greater mean yellow and red readings than did LLSBO (5 yellow, 0.5 red) and LLSBOW (4 yellow, 0.4 red), respectively (data not shown). But the pigment decomposition rate was not dependent upon the effect of 18:3 concentration on color

changes. The initial differences disappeared when all the oils became too pale to be read by the equipment at the end of 12-month storage. TBHQ addition had no effect on the yellow and red color changes of the SBO. The speed of pigment decomposition was greater at 32°C than at 21°C.

Sensory evaluations. Initially, LLSBO and LLSBOW had significantly better overall flavor quality scores than did ULSBO and ULSBOW, respectively (Table 3). At 2-month storage, significant differences disappeared and the ULSBO tended to have better overall flavor quality later in storage, especially at 21°C. Similar trend was observed in the change of PV of the oils demonstrating that ULSBO was more stable than LLSBO despite the initial more oxidized level of ULSBO than LLSBO due to processing. Generally, oils stored at 21°C had better overall flavor quality than did oils stored at 32°C with the same 18:3 concentration and TBHQ level, especially as storage time increased. The TBHQ addition tended to have a negative effect on overall flavor quality by sensory evaluations, especially through 8 months of storage. By 10 and 12 months, however, TBHQ addition tended to enhance overall oil quality scores.

To further evaluate the impact of TBHQ on oil flavor, an untrained 33-member panel was used to compare the overall flavor characteristics of fresh commercial SBO without TBHQ addition to that of fresh commercial SBO with 100 ppm and to that of fresh commercial SBO with 200 ppm TBHQ addition by triangle test. No difference was found between the overall flavor characteristics of SBO without TBHQ addition and SBO with either 100 ppm or 200 ppm TBHQ addition. More extensive sensory evaluations might reveal more information on the impact of TBHQ on oil flavor. A previous study on the effect of TBHQ on oil flavor stability found that TBHQ treatment did not enhance the flavor stability of oils (18).

For individual flavors, the predominant attributes detected by panelists in the SBO included painty, fishy, grassy, beany, nutty, and buttery flavors. The Pearson correlation coefficients between the intensity of painty, fishy, grassy, beany, nutty, and buttery flavors and overall oil quality scores were 0.870, 0.731, 0.687, 0.681, 0.403, and 0.002, respectively. That is, the intensity of painty, fishy, grassy and beany flavors had strong correlations with overall oil quality scores in sensory evaluations, whereas the intensity of nutty and buttery flavors had weak or no correlations with overall flavor quality. The sensory evaluation data of SBO with overall oil quality and multiple individual flavors represent typical multivariate data. Interpretation of the effects of 18:3 concentration, TBHQ addition, and temperature on individual flavor intensities and integrating the impact of individual flavor on overall sensory characteristics of SBO is beyond the scope of this paper; however, a more sophisticated method to simplify the representation of sensory characteristics of SBO is in process.

In general, flavor scores paralleled those of the objective test results, in showing a slight advantage in stability and flavor quality, especially over time, of ULSBO over LLSBO. The results showed a further advantage of UL oil, in that, despite lower total tocopherol and tocopherol homologue concentrations in the initial and finished oils, UL still emerged as better oil.

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Table 1

Fatty Acid Composition (area %), Calculated Oxidizability[a], Iodine Value,[b] and Totox Value[c] of Soybean Oils (SBO) with Low and Ultra-Low Linolenic Acid (18:3) Concentrations

Oils[d]	Fatty Acid Methyl Esters					Oxidizability	Iodine value	Totox value	
	16:0 (Palmitic)	18:0 (Stearic)	18:1 (Oleic)	18:2 (Linoleic)	18:3 (Linolenic)			Before	After
LLSBO	11.1	5.0	23.0	58.7	2.2	6.8	127.2	6.1	55.9
ULSBO	11.4	5.0	25.2	57.4	1.0	6.4	123.7	2.8	43.6

[a] Oxidizability = [oleate% + 10.3 (linoleate%) + 21.6 (linolenate%)]/100 (Ref. 1).

[b] Iodine values were calculated from the FAME profile, according to AOCS Official Method Cd 1c-85 (Ref. 11).

[c] Totox value = [p-AV + 2 PV] (Ref. 16) of SBO initially and at the end of 12-month storage; the values are the means of all LLSBO or all ULSBO, regardless of the level of TBHQ addition and storage temperature.

[d] LLSBO = SBO with low-18:3 concentration; ULSBO = SBO with ultra-low-18:3 concentration.

Table 2. Tocopherol Concentrations (ug/g) of Soybean Oils Before and After Storage[a]

Oil[b]	Tocopherol homologue						Total		Total	%
	α		γ		δ					
	Before	After	Before	After	Before	After	Before	After	loss[c]	Loss
LLSBO21	249	221	402	343	120	104	770	668	$102^{a,b}$	13^{a}
LLSBOW21	280	235	396	348	117	107	793	689	$104^{a,b}$	13^{a}
ULSBO21	125	104	204	209	35	31	364	344	20^{c}	6^{b}
ULSBOW21	125	103	210	194	36	32	372	329	42^{c}	$11^{a,b}$
LLSBO32	249	248	402	347	120	100	770	695	$75^{b,c}$	$10^{a,b}$
LLSBOW32	280	237	396	344	117	102	793	684	109^{a}	14^{a}
ULSBO32	125	119	204	192	35	28	364	339	25^{c}	7^{b}
ULSBOW32	125	98	210	195	36	31	372	324	$47^{d,c}$	13^{a}
Comparison[d]										
LLSBO	264	235	399	346	119	103	782	684	98^{a}	13^{a}
ULSBO	125	106	207	197	36	31	368	334	34^{b}	9^{a}
W/O TBHQ	187	173	303	273	77	66	567	511	$56^{a,b}$	10^{a}
W TBHQ	202	168	303	270	77	68	582	507	$76^{a,b}$	13^{a}
21°C	195	166	303	273	59	53	575	508	$67^{a,b}$	11^{a}
32°C	195	151	303	270	59	50	575	510	$64^{a,b}$	11^{a}

[a] Individual and total tocopherol concentrations of SBO before and after 12-month storage. The values are averages of duplicate analyses, with the overall mean of STDEV at 4.1.

[b] Refer to footnote d in table 1 for definitions of LLSBO and ULSBO. Presence of W means with TBHQ; absence of W = without TBHQ; 21 or 32 refers to storage temperature in degree C.

[c] Values in the same column with supercripts in common were not significantly different ($p < 0.05$).

[d] Comparison of the means at two levels of one treatment factor, averaged over the levels of the other two factors.

Table 3

Oil Stability Indices (h), Peroxide Values (meq/kg), p-Anisidine Values (mmol/kg), Polar Compound Percentages (%), and Sensory Evaluations for Overall Oil Quality of Soybean Oils[a] with Low and Ultra-Low Linolenic Acid Concentrations

Analysis[b]	Soybean oil	Storage time (month)						
		0	2	4	6	8	10	12
OSI	LLSBO21	6.9^{c}	4.9^{b}	4.8^{d}	4.1^{d}	4.0^{d}	3.9^{c}	3.8^{c}
	ULSBO21	5.2^{c}	4.2^{b}	3.6^{d,e}	3.3^{d,e}	3.1^{e}	2.9^{c}	2.8^{c}
	LLSBOW21	17.4^{b}	15.8^{a}	12.6^{b}	11.8^{b}	11.5^{a}	11.0^{a}	10.7^{a}
	ULSBOW21	20.7^{a}	15.9^{a}	14.0^{a}	13.2^{a}	11.8^{a}	11.8^{a}	11.3^{a}
	LLSBO32	6.9^{c}	4.6^{b}	4.6^{d}	4.0^{d}	3.7^{d,e}	3.4^{c}	3.2^{c}
	ULSBO32	5.2^{c}	4.1^{b}	3.3^{e}	3.1^{e}	3.0^{e}	2.7^{c}	2.4^{c}
	LLSBOW32	17.4^{b}	15.1^{a}	9.2^{c}	10.0^{c}	9.4^{c}	8.6^{b}	8.1^{b}
	ULSBOW32	20.7^{a}	16.2^{a}	12.6^{b}	11.7^{b}	10.4^{b}	8.8^{b}	8.4^{b}
PV	LLSBO21	0.3^{b}	1.5^{c}	3.1^{b,c,}	3.4^{e}	8.4^{c,d}	15.0^{b}	27.3^{a}
	ULSBO21	0.4^{a}	3.6^{a}	4.6^{b}	4.8^{c}	10.5^{b,c}	11.5^{c,d}	20.8^{b}
	LLSBOW21	0.3^{b}	1.1^{c}	1.7^{d}	1.8^{e}	4.1^{e}	7.1^{d}	8.3^{d}
	ULSBOW21	0.2^{b}	1.5^{c}	2.0^{d}	2.1^{e}	7.0^{c,d,}	8.5^{d}	9.7^{d}
	LLSBO32	0.3^{b}	2.8^{b}	3.7^{b,c}	6.8^{a}	14.5^{a}	20.0^{a}	29.3^{a}
	ULSBO32	0.4^{a}	4.3^{a}	7.7^{a}	7.9^{a}	13.4^{a,b}	14.0^{b,c}	25.1^{a,b}
	LLSBOW32	0.3^{b}	1.5^{c}	3.9^{b,c}	4.0^{d}	7.6^{c,d,e}	13.4^{b,c}	14.5^{c}
	ULSBOW32	0.2^{b}	1.4^{c}	2.7^{c,d}	3.4^{e}	3.4^{d,e}	9.6^{d}	12.7^{c,d}

[a] See footnote d in Table 1 and footnote b in Table 2 for definitions of SBO treatments.

[b] Values in the same column for each test with supercripts in common were not significantly different ($p < 0.05$).

[c] Overall oil quality score is based on the scale: 10=excellent, 9 and 8=good, 7 and 6=fair, 5 and 4=poor, 3, 2 and 1=very poor.

Table 3 (continued)

Analysis[b]	Soybean oil	Storage time (month)						
		0	2	4	6	8	10	12
p-AV	LLSBO21	5.5^{a}	7.2^{b}	7.3^{c}	7.5^{b}	8.0^{b}	12.9^{b}	13.0^{c}
	ULSBO21	2.3^{b}	2.9^{d}	3.0^{f,g}	3.0^{e}	4.2^{d,e}	7.3^{c}	8.8^{d}
	LLSBOW21	5.5^{a}	7.5^{b}	6.5^{d}	7.0^{c}	6.9^{b,c}	13.7^{b}	9.8^{d}
	ULSBOW21	2.0^{b}	2.4^{d}	2.4^{g}	2.5^{f}	2.8^{e}	5.6^{c}	3.9^{e}
	LLSBO32	5.5^{a}	8.5^{a}	9.3^{a}	12.1^{a}	12.3^{a}	19.1^{a}	27.0^{a}
	ULSBO32	2.3^{b}	3.9^{c}	5.5^{e}	6.1^{d}	7.1^{b,c}	14.2^{b}	17.1^{b}
	LLSBOW32	5.5^{a}	7.8^{a,b}	8.0^{b}	7.5^{b}	6.7^{b,c,d}	18.9^{a}	14.8^{c}
	ULSBOW32	2.0^{b}	2.9^{d}	3.1^{f}	3.0^{e}	4.8$^{c.d.e}$	13.9^{b}	7.9^{d}
Polar compound percentages	LLSBO21	2.6^{a}	2.9^{d}	3.5^{b}	3.7^{b,c,}	4.0^{b,c}	4.1^{b}	4.2^{b}
	ULSBO21	2.5^{a}	3.0^{d}	3.1^{c}	3.4^{d}	3.5^{c,d}	3.4^{c}	3.9$^{b.c}$
	LLSBOW21	2.2^{a}	3.0^{c}	3.1^{c}	3.8^{b,c}	4.2^{a,b}	4.3^{b}	4.1^{b}
	ULSBOW21	2.6^{a}	2.9^{d}	2.9^{c}	3.5^{c,d}	3.6^{d}	3.5^{c}	4.2^{b}
	LLSBO32	2.6^{a}	3.3^{a}	4.0^{a}	3.9^{b}	4.7^{a}	4.8^{a}	4.7^{a}
	ULSBO32	2.5^{a}	3.1^{b}	3.7^{a,b}	3.9^{b}	4.2^{a,b}	4.3^{b}	4.2^{b}
	LLSBOW32	2.2^{a}	3.3^{d}	3.8^{a,b}	4.1^{a}	4.0^{b,c}	4.1^{b}	4.3^{b}
	ULSBOW32	2.6^{a}	3.2^{d}	3.5^{b}	3.6^{b,c,d}	3.6^{c,d}	3.7^{c}	3.6^{c}
Sensory for overall oil quality[c]	LLSBO21	8.4^{a}	7.5^{a}	7.5^{a,b}	5.5^{a,b}	5.2^{a,b}	4.9^{a}	3.2^{a}
	ULSBO21	7.8^{b}	7.5^{a}	7.5^{a}	5.7^{a,b}	5.7^{a}	4.1^{a,b,c}	3.4^{a}
	LLSBOW21	8.4^{a}	7.5^{a}	6.9^{a,b}	6.3^{a}	4.9^{a,b,c}	3.5^{a,b,c}	3.3^{a}
	ULSBOW21	7.7^{b}	6.8^{a}	6.6^{a,b,}	5.2^{a,b}	4.8^{a,b,c}	4.4^{a,b}	3.4^{a}
	LLSBO32	8.4^{a}	7.2^{a}	6.2^{b,c}	5.1^{a,b}	4.2^{b,c}	3.6^{a,b,c}	3.3^{a}
	ULSBO32	7.8^{b}	7.2^{a}	6.6^{a,b,c}	5.4^{a,b}	4.5^{b,c}	2.9^{c}	2.7^{a}
	LLSBOW32	8.4^{a}	7.1^{a}	5.5^{c}	5.1^{a,b}	4.1^{b,c}	3.7^{a,b,c}	2.7^{a}
	ULSBOW32	7.7^{b}	7.1^{a}	6.3^{a,b,c}	4.9^{b}	4.1^{c}	3.0$^{b.c}$	3.2^{a}

Multivariate Sensory Characteristics of Low and Ultra-Low Linolenic Soybean Oils Displayed as Faces

A paper reviewed and recommended for publication upon revision in the Journal of American Oil Chemists' Society

Caiping Su[a], Monoj Gupta[b] and Pamela White[a,]*

[a]Food Science and Human Nutrition Department and Center for Crops Utilization Research, Iowa State University, Ames, IA 50011

[b]MG Edible Oil Consulting Intl, Richardson, TX 75080

Running Title: USE OF CHERNOFF FACES

* To whom correspondence should be addressed at 2312 Food Sciences Building, Iowa State University, Ames, IA 50011-1060. Phone: 515-294-9688, Fax: 515-294-8181, Email: pjwhite@iastate.edu

ABSTRACT: The effects of linolenic acid (18:3) concentration, combined with TBHQ addition, temperature and storage time, on the flavor stability of soybean oils were evaluated. A descriptive panel was trained to evaluate the overall oil quality and the intensity of individual flavors of soybean oils during 12-month storage under fluorescent light at both

21°C and 32°C. Chernoff faces were used to achieve a simplified and integrated interpretation of the multivariate sensory data and to facilitate the interpretation of the vast amount of the data. When fresh, soybean oil (SBO) with low 18:3 (2.2% 18:3, LLSBO) showed better flavor stability than did SBO with ultra-low 18:3 (1.0% 18:3, ULSBO). This trend disappeared during storage. During 10- to 12-month storage, a painty flavor became predominant in all oils, which may have made it difficult for panelists to detect differences in treatment effect on flavor characteristics of soybean oils. During early storage, oils with TBHQ addition had poorer overall oil quality and stronger beany, painty and fishy flavors than did oils without TBHQ addition. This trend disappeared as storage time progressed to 10 months. Oils stored at 32°C had poorer overall oil quality, and stronger painty, fishy and beany flavors than did oils stored at 21°C starting from 2-month storage.

KEY WORDS: Chernoff faces, lipid oxidation, low-linolenic acid, multivariate data analysis, oil stability, *sensory evaluation*, soybean oil, ultra-low linolenic acid.

Soybean oil is very prone to flavor deterioration, and sensory evaluation provides the ultimate judgment of its flavor stability. The recommended practice of the AOCS is to evaluate overall oil quality and the intensity of individual flavors. The number of flavors that can be present in soybean oil can be as many as 15, or more (1). Therefore, the resulting data are multivariate, because they are made up of complex interrelated elements. The standard display of data, such as numbers, may obscure the recognition of relationships among elements. To make overall perception and interrelationships immediately apparent, and to provide a more accurate judgment as a well-integrated pictorial display, the multivariate data

analysis methods may be used. There are reports of the use of multivariate data analysis methods, such as principal component analysis (PCA), factor analysis, and generalized procrustes analysis in the sensory evaluation of different food products (2, 3, 4); however, the use of Chernoff faces to characterize sensory evaluation of food products or soybean oil was not found in the literature.

This paper focuses on the descriptive sensory analysis of soybean oil flavor stability and the use of Chernoff faces (5) to simplify the interpretation and graphically display an abundant amount of sensory data. This method involves letting the size, shape, or orientation of each feature of a cartoon face represent a particular variable (overall flavor quality or the individual flavor descriptor in the current work) (6). Thus, one might let the area of the face represent overall flavor quality of the oil, the shape of the face a fishy flavor, the length of the nose a third characteristic, and so on. Programs have been developed that allow the representation of up to 15 (7) or 20 variables (8). It is these characteristics that inspired the authors to explore the use of Chernoff faces.

The specific objectives of the current work were to report the sensory evaluation, by using Chernoff faces, of soybean oils with low–linolenic acid (18:3, ~2.2 %) and ultra–low–18:3 concentrations (~1.0 %), with and without the addition of TBHQ, and at two storage temperatures (21 °C and 32 °C) during storage for 12 months. A related paper (9) gave complete information on the physical, chemical and general sensory tests used to assess these oil treatments.

MATERIALS AND METHODS

Soybean oils and design. Soybeans (*Glycine max*) with low-18:3 (LL, 2.2%) and ultra-low-18:3 (UL, 1.0%) concentrations, grown in summer 2000 in Iowa (weather zone 2), were obtained from Protein Technologies, Inc. (St. Louis, MO). The LL soybeans were crushed by the Montana Power Group in Culverston, Montana, and the UL soybeans were crushed at the POS Pilot Plant Corporation in Saskatoon, Saskatchewan, Canada. Both oils were hexane-extracted, and refined, bleached, deodorized, and bottled at the POS Plant. Citric acid (50 ppm) was added to the oils during the cool-down stage of deodorization. The antioxidant, TBHQ (100 ppm), was added to half of each oil type at the deodorization step before bottling in co-extruded polyethylene terephthalate (PET) plastic bottles. The bottles were sparged with nitrogen until they contained less than 2% oxygen in the headspace, then sealed. Bottled oils were sent to Iowa State University (ISU, Ames, IA) for evaluation. Thus, four SBO treatments were tested, including low-18:3 SBO (LLSBO), LLSBO with the addition of 100 ppm TBHQ (LLSBOW), ultra-low-18:3 SBO (ULSBO), and ULSBO with the addition of 100 ppm TBHQ (ULSBOW). The LLSBO and LLSBOW contained 11.1% palmitic acid, 5.0% stearic acid, 23.0% oleic acid, 58.7% linoleic acid, and 2.2% linolenic acid. The ULSBO and ULSBOW contained 11.4% palmitic acid, 5.0% stearic acid, 25.2% oleic acid, 57.4% linoleic acid, and 1.0% linolenic acid. For each of these four treatments, two bottles were retained at arrival, and half of the remaining bottles were stored under fluorescent light with uniform exposure of 70-foot candle light intensity at 21°C and the other half at 32°C, respectively, for 12 months. Thus, there were eight treatments during storage. Duplicate bottles of oil from each treatment were analyzed in duplicate at 0, 2, 4, 6, 8, 10, and 12 months of storage for flavor characteristics.

Chemical and other objective evaluation methods. A related paper presents complete information on the impact of 18:3 content, TBHQ addition, storage temperature and storage time on PV, oil stability index, p-anisidine value, polar compounds, and Lovibond colors, including statistical evaluations of the differences (9).

Fatty acid composition by GC. Fatty acid compositions of SBO were determined by converting TAG into FAME according to a method described by Hammond (10). The GC conditions were the same as described by Shen *et al.* (11).

Sensory evaluations. The sensory evaluations were conducted according to AOCS Recommended Practice Cg 2-83 (1) as described elsewhere (9).

Faces. Statistical software S-plus 6.0.3 Release 2 for Microsoft Windows was used to draw the faces (7). In this software, the facial features and their sequences are: 1-area of face; 2-shape of face; 3-length of nose; 4-location of mouth; 5-curve of smile; 6-width of mouth, and so on (Table 1). Thus, the area of the face represents the value of the first variable (flavor attribute, in this case); the shape of the face represents the second flavor attribute, and so on. The researcher can perform permutation by arranging the order of flavor attributes in the data table to get the best-represented data by the faces. Also, all facial features do not need to have a variable assigned. After several attempts of permutation to assign flavor attributes to different facial features, we decided upon the correspondence between flavor attributes and facial features shown (Table 1). The range in the dimensions and/or shape of each facial feature was from 1 to 10, with 10 representing "excellent" and 1 representing "poor" for each of the flavor attributes. We chose not to assign an attribute to the length of the nose (dimension # 3, Table 1), and to dimensions # 7 through # 15; thus, the computer program assumed the mid-value of 5 for these unassigned facial features. The numerical data supplied

by the sensory panelists for each attribute were used by the statistical program to draw a face representing the sensory evaluation of a specific oil at a specific time. The data of all flavor attributes of a specific oil at a specific time, then, makes up the "face" for that oil at that time.

The S-plus command was designated as follows: faces(as.matrix(faces1), labels=row.names(faces1), nrow=4, ncol=8). The term, "faces" is the command to draw a face plot; "as.matrix" defines the data table to be used by faces command; "faces1' is the name of the data table to be used by the faces command; "labels= row.names(faces1)" means that each of the faces will be labeled by the row name of data table faces1; and "nrow=4, ncol=8" means there will be 4 rows and 8 columns of faces displayed on one page as shown in Figures 1.

Statistical Analyses. The Pearson correlation coefficients between the intensity of individual flavors and overall flavor quality scores of SBO were calculated by using SAS software (12).

RESULTS AND DISCUSSION

Overall flavor quality. Initially, LLSBO and LLSBOW had better overall oil quality than did ULSBO and ULSBOW, respectively (data summarized in Table 2 from reference (9). The differences tended to reverse as storage progressed, with both UL treatments having better overall scores in later months of storage. This observation was consistent with the results for the PV of the oils (9). That is, when TBHQ was absent and at the same storage temperature, the ULSBO initially had significantly greater PV than did LLSBO. But the trend reversed during storage by 10 months at 21°C and by 8 months at 32°C (9). The TBHQ addition tended to have a negative effect on overall oil quality by sensory evaluations, especially

through 8 months of storage. By 10 and 12 months, however, TBHQ addition tended to minimize the poor overall oil quality scores, likely because of its ability to retard lipid oxidation (9). Generally, oils stored at 21°C had better overall flavor quality than did oils stored at 32°C with the same linolenic acid and TBHQ level, especially as storage time increased between 4 and 10 months. The overall appearance of the faces in figures 1 illustrate these quality differences at a glance.

Intensity of individual flavors. The individual flavors detected by panelists in oils included nutty, buttery, corny, beany, hydrogenated, burned, weedy, grassy, rubbery, melon, painty, fishy, bitter taste, astringency, rancid and oxidized. The predominant attributes (i.e. those attributes detected by at least 3 panelists in one session for at least 5 sessions throughout the evaluation time) detected by panelists in the soybean oils included painty, fishy, grassy, beany, nutty, and buttery flavors. The Pearson correlation coefficients between the intensity of each of these flavors and overall flavor quality scores were 0.870, 0.731, 0.687, 0.681, 0.403, and 0.002, respectively. That is, the more intense (lower values) the flavors of painty, fishy, grassy and beany flavors, the poorer (lower) the overall flavor quality scores in sensory evaluations. There were no correlations between the intensity of nutty and buttery flavors and overall flavor quality scores.

Faces. Each face in fig. 1 represents both the overall oil quality as well as the intensity of individual flavors of one oil treatment at a specific storage time. In other words, it is a highly condensed version of the data. The faces can be used to compare treatment impact on flavor characteristics of soybean oils. Initially, faces representing LLSBO were more "happy" and round than faces of ULSBO. The differences between the LL and UL SBO tended to disappear at about 4-month storage. The faces representing SBO with TBHQ addition were

less "happy" than faces of SBO without TBHQ addition through 8 months of storage, and this difference tended to disappear at 10-month storage. Generally, faces of oils stored at 32 °C were closer to "poor" than faces of oils stored at 21 °C, and this difference became clearer at 8-month storage.

The faces can also be used to detect, at a glance, the time point at which an individual soybean oil changed its multivariate sensory characteristics from relatively "excellent" to "poor". For example, initially, all the faces representing oils at arrival (0-month storage) were very close to the excellent example. Even so, faces of ULSBO and ULSBOW were not as "happy" as the faces of LLSBO and LLSBOW. At 2-month storage, faces were less round and began to develop features that were less "happy" than faces at 0-month storage time. If the face of 4-LLSBOW32 (SBO with LL concentration, 100 ppm TBHQ addition, stored at 32°C and at 4-month storage time) was viewed simply as an outlier, the 8-month storage time seems to be when the faces began to turn "poor" as demonstrated by the consistently smaller, thinner, and longer face, the downward curvature of the smile, the longer distance between the nose and mouth, and the smaller width of the mouth. The faces at 6-month storage were in transition from good to bad. By the end of 12-month storage, all the faces of oils were very close to the "poor" example.

Both flavor quality scores and multiple individual flavors for the soybean oils represent typical multivariate data. The overall, combined sensory characteristics of soybean oils, however, represent an integrated perception. If the data of the intensity of all individual flavors were presented in the same way as the flavor quality scores in Table 2, one would need at least four more similar-sized tables. Repetitious viewing of large tables of data is tedious as described by the two 19th century economists, Jocob and Howard, "Getting

information from a table is like extracting sunlight from a cucumber" (13, 14). Thus, to improve data interpretation, the method of Chernoff faces was used to represent the multi-factored changes of flavor characteristics of soybean oils during storage in a straight-forward pictorial display.

The method of using Chernoff faces in other applications has been criticized, because of the effect associated with a particular subjective facial feature; for example, curvature of the smile and/or other certain facial features may be more informative than other features (5, 15). A wisely chosen featural assignment, however, limits this possibility. In our case, for example, the painty, fishy, and beany flavors, significant off-flavors associated with soybean oil flavor instability, were assigned to the shape of the face, curvature of the smile, and width of mouth, respectively, which have major impact on facial expressions. In other studies, where there are no major attributes, the assignment of a variable to a more informative facial feature can be avoided. Therefore, the permutation of the variable assignment to a facial feature, as performed in this application, is necessary to get the best data representation by the faces.

Finally, the disadvantage of subjectivity, which is sometimes noted when using Chernoff faces, actually may be an advantage when applied to sensory evaluation analyses. To the consumer, excellent sensory quality of a food product makes them "happy". This paper demonstrates the use of Chernoff faces as an effective procedure for researchers to simplify the presentation of sensory characteristics of edible oils, and to obtain an integrated judgment of the overall flavor characteristics of soybean oils at a glance. People react quickly to faces; thus, we envision the popularity of Chernoff faces in the sensory evaluation of a variety of food products, as well as other applications described by other authors (14).

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Table 1
Correspondence between the Assigned Facial Features and the Numerical Values Assigned to the Facial Features

Dimension	Facial feature	Flavor attribute[a]	Numerical value assigned[b]
1	Area of face	Overall oil quality	1-10 from sensory data
2	Shape of face	Painty	1-10 from sensory data
3	Length of nose	-	5
4	Location of mouth	Grassy	1-10 from sensory data
5	Curve of smile	Fishy	1-10 from sensory data
6	Width of mouth	Beany	1-10 from sensory data
7	Location of eyes	-	5
8	Separation of eyes	-	5
9	Angle of eyes	-	5
10	Shape of eyes	-	5
11	Width of eyes	-	5
12	Location of pupil	-	5
13	Location of eyebrow	-	5
14	Angle of eyebrow	-	5
15	Width of eyebrow	-	5

[a] The sign "-" means no flavor attribute was assigned to that facial feature and S-plus assumes a mid-value of "5" to that feature to draw a complete face.

[b] Values of 10 (excellent) to 1 (poor) were given to each flavor characteristic of oils, according to the panelists' scores.

Table 2

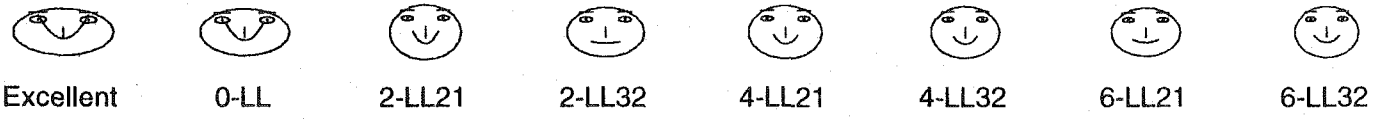
Sensory Evaluation Scores[a] of Overall Quality of Soybean Oils

Oils[b]	Storage time (Month)						
	0	2	4	6	8	10	12
LLSBO21	8.4	7.5	7.5	5.5	5.2	4.9	3.2
ULSBO21	7.8	7.5	7.5	5.7	5.7	4.1	3.3
LLSBOW21	8.4	7.5	6.9	6.3	4.9	3.5	3.4
ULSBOW21	7.7	6.8	6.6	5.2	4.8	4.4	3.4
LLSBO32	8.4	7.2	6.2	5.1	4.2	3.6	3.3
ULSBO32	7.8	7.2	6.6	5.4	4.5	2.9	2.7
LLSBOW32	8.4	7.1	5.5	5.1	4.1	3.7	2.7
ULSBOW32	7.7	7.1	6.3	4.9	4.1	3.0	3.2
Comparison[c]							
LLSBO	8.4	7.3	6.5	5.5	4.6	3.1	3.1
ULSBO	7.7	7.1	6.8	5.3	4.8	3.6	3.2
W/O TBHQ	8.1	7.3	7.0	5.4	4.9	3.9	3.1
W TBHQ	8.0	7.1	6.3	5.4	4.5	3.7	3.1
21°C	8.1	7.4	7.1	5.7	5.1	4.2	3.3
32°C	8.1	7.1	6.1	5.1	4.2	3.3	3.0

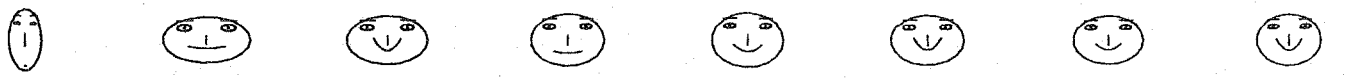
[a] A score of 10 = excellent, a score of 1 = very poor (Ref 1).

[b] Refer to footnote b in table 1 for definitions of LLSBO and ULSBO. Presence of W = with TBHQ; absence of W = without TBHQ; 21 or 32 refers to storage temperature at 21 and 32°C.

[c] Comparison of the means at two levels of one treatment factor regardless of the levels of the other two factors.

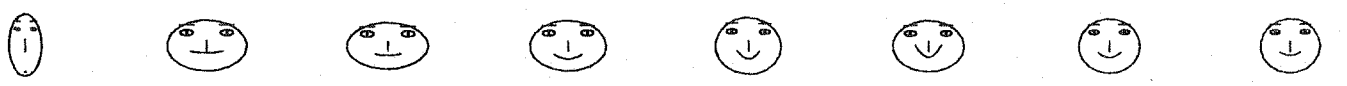


Excellent 0-LL 2-LL21 2-LL32 4-LL21 4-LL32 6-LL21 6-LL32



Bad 0-UL 2-UL21 2-UL32 4-UL21 4-UL32 6-UL21 6-UL32

Excellent 0-LLW 2-LLW21 2-LLW32 4-LLW21 4-LLW32 6-LLW21 6-LLW32



Bad 0-ULW 2-ULW21 2-ULW32 4-ULW21 4-ULW32 6-ULW21 6-ULW32


8-LL21


8-LL32


10-LL21


10-LL32


12-LL21


12-LL32


Excellent


8-UL21


8-UL32


10-UL21


10-UL32


12-UL21


12-UL32


Bad


8-LLW21


8-LLW32


10-LLW21


10-LLW32


12-LLW21


12-LLW32


Excellent


8-ULW21


8-ULW32


10-ULW21


10-ULW32


12-ULW21


12-ULW32


Bad

Figure 1. Faces representing the two extreme examples and the sensory characteristics of soybean oils (SBO) during storage. Excellent and poor examples are given in the first and last column. LL = SBO with low-linolenic acid (18:3); UL = SBO with ultra-low 18:3; Presence of W means SBO with 100 ppm TBHQ addition; The 0, 2, 4, 6, 8, 10, and 12 means SBO stored for 0 (fresh), 2, 4, 6, 8, 10, and 12 months, respectively; The 21 and 32 = storage temperature at 21°C and 32 °C, respectively.

Optimizing Oleic Acid Composition in Frying Soybean Oils for Maximal Health Benefit and Best Oxidative Stability

A paper to be submitted to the Journal of American Oil Chemists' Society

Caiping Su and Pamela White*

Department of Food Science and Human Nutrition and Center for Crops Utilization Research, Iowa State University, Ames, Iowa 50011

Running Title: STABILITIES OF HIGH-OLEIC SOYBEAN OILS

* To whom correspondence should be addressed at 2312 Food Sciences Building, Iowa State University, Ames, IA 50011-1060. Phone: 515-294-9688, Fax: 515-294-8181, Email: pjwhite@iastate.edu

ABSTRACT: The objective of this project was to determine the optimum percentage of oleic acid (OA) in soybean oils (SBO) that could be achieved by blending high-oleic (HO, 79% OA) and control (conventional SBO, 21.5% OA) to obtain maximum frying stability while retaining good flavor potential. The control and HO SBO were tested as is, as well as blended in different ratios to make three blended oils containing 36.9%, 50.7%, and 64.7% OA, abbreviated as 37%OA, 51%OA, and 65%OA, respectively, in addition, a low-linolenic (LL) SBO contained 1.4% linolenic acid and 25.3% OA). White bread cubes (8.19 cm^3) were fried in each of eighteen oils (6 treatments × 3 replicates). In general, the results suggested

that the overall stability of the six oil treatments from the best to the poorest was: 79%OA, 65%OA, 51%OA, LL ≥ 37%OA, and Control , as measured by the oil stability index, conjugated dienoic acid concentration, viscosity, polar compound percentage and Hunter Lab colors of the fried oils, and PV of the oil extracted from fresh and stored bread cubes.

KEY WORDS: Conjugated dienoic acid, fried bread cubes, free fatty acids, frying, heat stability, *high-oleic acid soybean oil*, low-linolenic acid soybean oil, oxidative stability, polar compounds, viscosity.

Soybean oil (SBO) has a good nutritional profile because of its high proportion of unsaturated FA, but the oil has poor oxidative stability and is prone to flavor deterioration. The fatty acids, linoleic (18:2) and linolenic acid (18:3) in SBO oxidize quickly and are the major contributors of the poor stability of SBO (1, 2). To improve oxidative and flavor stability, the SBO may be hydrogenated to reduce the concentration of PUFA (and increase the saturated FA); however, *trans* fatty acids (*t*FA) are formed and saturated fatty acids are increased during this process. Because consumption of a diet high in *trans* FAs has been reported to raise total and low-density lipoprotein (LDL)-cholesterol and lower high-density lipoprotein (HDL)-cholesterol levels (3) and a diet high in ratio of saturated fatty acids to PUFA has been shown to increase serum total cholesterol (4), indicators of increased cardiovascular risk, lowering the 18:3 content to a level similar to that obtained by partial hydrogenation, but without *trans* formation, has been an objective of plant breeders. The SBO with different lowered levels of 18:3 have been developed and studied (5, 6, 7). The oxidative and flavor stabilities of SBO containing as low as 1.0% 18:3 were compared to

SBO containing 2.2% 18:3 in a previous study (6, 7). The 1.0% 18:3 oil was more stable than the 2.2% 18:3 oil by oxidative and flavor stability indices. On the other hand, the 18:3 is an essential fatty acid belonging to a group of fatty acids called omega-3 fatty acids, which reduce or help prevent certain chronic diseases (8). Thus, reducing 18:3 to a minimal level may diminish the health benefits of SBO. Therefore, developing SBO with enhanced stability, but still retaining some 18:3, with no formation of *t*FA, and with a maximal amount of total unsaturated FA is desirable.

Studies have shown that the oxidation rate of OA is much slower than that of the PUFA, 18:2 and 18:3 (9). A diet high in monounsaturates may also help to reduce raised levels of total plasma cholesterol without reducing the HDL-cholesterol level (10). Therefore, the incentive to breed HO soybean (reducing, but not eliminating 18:2 and 18:3, reducing total saturated FA, and eliminating *t*FA) becomes obvious. Also such an oil would require no additional processing, thus could result in more profit for farmers and processors (11). The experiment in this study included control (conventional SBO, 21.5% OA) and high-oleic SBO (HO, 79% OA) which were tested as is, as well as blended in different ratios to make three blended oils containing 36.9%, 50.7%, and 64.7% OA, abbreviated as 37%OA, 51%OA, and 65%OA, respectively, in addition, a low-linolenic (LL) SBO contained 1.4% linolenic acid and 25.3% OA. One objective of this project was to determine the optimum percentage of OA in SBO that could be achieved by blending 79%OA and control to obtain maximum frying stability while retaining good flavor potential. It is a common belief that the blended oils can be only as stable as the "poorest" oil. A second objective was to determine the impact of blending a relatively unstable control SBO with a highly stable HO SBO.

MATERIALS AND METHODS

SBOs and design. Soybeans (*Glycine max*) with high-oleic acid (HO, 79% OA), low-linolenic acid (LL, 1.4% with 25.3% OA), and conventional (control, 21.3% OA) fatty acid compositions, grown in summer 1998 in Iowa (weather zone 2), were obtained from Protein Technologies, Inc. (St. Louis, MO). The soybeans were crushed and the oils were hexane-extracted, in triplicate, in the Pilot Plant of the Center for Crops Utilization Research, Iowa State University (ISU), Ames, Iowa, following a previously published method (11). All the oils were refined and bleached following AOCS official methods Ca 9a-52, and Cc 8a-52, respectively, (12), and deodorized following the procedure described by Stone and Hammond (13). Triplicate sets of each oil were refined, bleached, and deodorized separately. Citric acid (100 ppm) was added to the oils during the cool-down stage of deodorization before placement in high-density polyethylene (HDPE) plastic bottles. The bottles were sparged with nitrogen, then sealed and stored at -10°C until used for testing.

Six total SBO treatments were evaluated for frying stability, including the three SBOs just mentioned (Control, LL, and the 79%OA) plus three oil blends prepared as follows: 1) 75% of the Control and 25% of the HO (37%OA), 2) 50% of the Control and 50% of the HO (51%OA), and 3) 25% (by weight) of the Control and 75% of the HO (65%OA).

Frying. Eighteen frying sessions (three simultaneous sessions in one day) were carried out (six oil treatments evaluated in triplicate). At each frying session, 220 g of an oil treatment was weighed into a Teflon-coated 473-mL electric baby fryer (National Presto Industries Inc., Eau Claire, Wisconsin) and the oil was then heated to 185°C within 10 min. The oil was heated at 185 ± 5°C for 2.5 h before frying. Eight 5-piece batches of crust-free bread cubes (2.54 cm x 2.54 cm x 1.27 cm) were fried for 1 min per batch at 3-min intervals.

Therefore, the actual frying of the cubes was completed within 0.5 h. The fried bread cubes were then drained and cooled to room temperature. Half of the bread cubes was used immediately for testing including evaluating PV of the extracted oil. The other half of the bread cubes was stored, loosely covered, at 60°C in the dark for 3 days before evaluating PV of the extracted oil by the same procedure used on fresh bread cubes. The oil remaining in the fryer was maintained at 185 ± 5°C for another 7 h for a total of 10 h heating on day 1, then cooled to 25°C. The oil was heated at 185 ± 5°C for another 10 h on day 2. Aliquots from each oil were taken before heating, immediately after frying, at the end of day 1 heating (10 h), and at the end of day 2 heating (20h).

Fatty acid composition by GC, tocopherol contents by HPLC, oil stability indices (OSI), and *polar compounds*. Fatty acid compositions of SBOs before frying were determined according to a method described by Hammond (14). The GC conditions were the same as described by Shen *et al.* (11). Calculated oxidizability and iodine value of the oils were determined according to formulas based on the fatty acid composition of the oils (9, 12). Tocopherol contents, the OSI, and the percentage of polar compounds of the oils before frying were determined according to AOCS Official Methods Ce 8-89, Cd 12b-92, and Cd 20-91, respectively (12). The HPLC conditions were the same as described elsewhere (6).

FFA. The percentage of FFA as OA of the frying oils was determined according to the AOCS Official Method Ca 5a-40 (12) as modified by Rukunudin *et al.* (15).

Viscosity. Viscosity of the oils before and after frying and heating was measured by using a Brookfield DV – II + viscometer (Brookfield Engineering Laboratories Inc., Stoughton, MA). One milliliter of oil was placed on the plate of the viscometer with cone spindle CP-42;

the viscosity of the sample was read in cP (1 cP = 1 mPa•s) directly from the viscometer maintained at 40°C by a circulating water bath.

Conjugated dienoic acid. The percentage of conjugated dienoic acid of the frying oils was determined according to the AOCS Official Method Ti 1a-64 (12) as a measurement of the diene conjugation of unsaturated linkages present in the fatty esters.

Colors. Colors of the frying oils were measured with a HunterLab colorimeter (Hunter Associate Laboratory, Inc., Reston, Virginia) at a 10° field of vision with illuminant D65. Oil (13.0g) was placed in a 60 × 15 cm standard disposable petri dish and the measurements were recorded in Hunter units of L (L = 0 (black), L = 100 (white)), a (+ a = red, − a = green), and b (+ b = yellow, − b = blue).

Peroxide values (PV) of the SBOs before frying and the extracted oil from bread cubes. The PV of the oils before frying was determined by the Stamm test as modified by Hamm *et al.* (16). Commercially available tetrachloroethane was purified as described elsewhere (6).

Oil from the fried bread cubes (3.0 g) was hexane-extracted as previously described (17). The extracted oil was used to determine the PV of the fried bread cubes by the same procedure as just mentioned.

Statistical analysis. There were 6 treatments × 3 replicates. The SAS full-way variance procedure was used to analyze the data (19). Differences in mean values among treatments were determined by the least significant difference test at $\alpha = 0.05$, unless listed otherwise.

RESULTS AND DISCUSSION

Fatty acid composition, calculated oxidizability, calculated iodine value (IV) (Table 1). The control oil had much greater palmitic (16:0), 18:2, and 18:3 acid concentrations than did the 79%OA, the blended treatments were intermediate in these FA levels, based on the ratios of each oil percent. The LL was similar in FA compositions to the control, except for its greatly reduced 18:3 level. Clearly, the calculated oxidizability and IV increased in the order: 79.1%OA, 65%OA, 51%OA, 37%OA, LL (25.3% OA), and control (21.5% OA). The greater the OA concentration in an oil, the lower the calculated oxidizability and IV. The effect of reduced linolenic acid concentration on the indices of calculated oxidizability and IV was not as great as the effect of elevated OA concentration.

Tocopherols (Table 1). There were no differences in the concentrations of total tocopherol concentrations among 79.1%OA, control and LL SBO, and any of their blends.

Oxidative stability indices (OSI) (Table 1). The OSI of all SBO treatments suggested an order of heat stability from greatest to lowest as: 79%OA, 65%OA, 51%OA, LL, 37%OA, control. These values are consistent with the predicted order by oxidizability and IV except for the LL treatment, which tended to be more stable than 37%OA as indicated by OSI instead of just slightly more stable than the control as predicted by calculated oxidizability and IV. The 65%OA (with 25% by weight the control blended in) had a big drop in OSI compared to 79%OA (Fig. 1 b), showing a trend of OSI that can not be predicted linearly by the OA concentrations in the oils. However, there were no differences in OSI values among 51%OA, 37%OA, control and LL.

FFA (Table 2). The FFA of all oil treatments increased as heating time increased. There were no significant differences in FFA among fresh SBOs and among the oils immediately after frying the bread cubes, except that the control had greater FFA than did LL immediately

after frying. Even though significant, the difference was small. At 10 and 20 h of heating, there tended to be greater FFA developed with increased 18:1 concentration of the oil, except for LL at 20 h. The greater 18:1 concentration, the greater the FFA. Previous researchers found the same trend in frying of potato chips (19). These findings were opposite from those of the OSI test. Perhaps this paradox was a result of a limitation of the method used. Generally, the oils that had greater 18:1 concentration were less viscous after 20 h frying, so the FFA may have been better dissolved in the alcohol used for titration of the FFA, resulting in a greater measured content than other, more viscous oils. The FFA is an important marker for oil quality. The recommended FFA in fresh refined, bleached, and deodorized oils is 0.05% maximum (20).

Viscosity (Table 2). Like the change of FFA in the frying oil treatments, the differences were small among fresh SBOs and among the oils immediately after frying the bread cubes. At 20 h heating, however, the oil viscosity increased with decreased 18:1 concentration, except that the LL was less viscous than 37%OA instead of the control as predicted by their 18:1 concentration order. This viscosity order suggests that the greater the 18:1, the more stable the oil was during frying, except for the LL treatment, whose very low 18:3 concentration simultaneously with its greater 18:1 elevated its stability above that of the 37%OA instead of just above that of the control as would be predicted solely by the 18:1 concentration order.

Conjugated dienoic acid (CDA) (Table 2). There were no differences in CDA among the fresh oils. Immediately after frying, and at 10 and 20 h heating, the greater the 18:1 concentration in the oils, the less the CDA formed during frying and heating, except that the LL treatment had less CDA than did 37%OA treatment. Again the LL treatment's very low

18:3 concentration along with its greater 18:1 percentage elevated its stability above that of the 37%OA.

Polar compounds. There were differences among oils in polar compound percentages only at 10 h heating with the greater the 18:1 concentration, the lower the polar compound formed during frying. Again the LL was very close in polar compound percentage to that of the 37%OA and the control. At 10-h heating, the polar compound percentages in all oils exceeded the maximum limit for used frying fats based on the German standard of 27% total polar compounds (21). At 20 h heating, the values were all similar, likely because the extensive breakdown in all oils had evened out. In this frying study, relatively small quantities of oil were used in each baby fryer, and only a small quantity of food was fried thus maintaining the amount of polar materials that are usually carried away by the fried food, which may have contributed to the great quantity of polar compounds in the frying oils.

Colors. There were increases in darkness, redness and yellowness in all oils as the length of heat treatment increased. The 79%OA was significantly less dark, red or yellow than the other oils at the end of 20-h heating, and there were no differences in darkness, redness, and yellowness among the other treatments indicating the 79%OA was the most heat stable oil among all treatments..

PV of the fresh oils and the oils extracted from the fried bread cubes. The order of PV from the least to the greatest in the fresh oils and in the oils extracted from fresh and stored bread generally was in reverse order of the 18:1 concentration in the oils except for the LL treatment. The reduced 18:3 concentration of the LL treatment elevated its stability above the order predicted by its 18:1 concentration in the fresh oil and stored bread cubes. The 79%OA

was the most stable oil and control was the least stable oil during storage of the fried bread cubes according to the PV.

Industry perceptions of blended oil quality would predict that the blended oils would be only as stable as the poorest oil blended in them. In actuality, the impact of blending on oil stability indices at 20 h heating was generally directly and linearly related to the % of control oil, and furthermore to the OA % for calculated oxidizability, IV, conjugated dienoic acid content, and viscosity (Figure 1. a – d). The impact of blending oils on the FFA, PV, and polar compounds was not linearly related to the ratios of the control in the blended oils but better than would be predicted based on percentage of the control (Fig 1 e - g). The OSI and HunterLab color values for the oils at 20 h heating showed that the presence of a small amount of the control in the blended oils greatly reduced the stability (Fig 1 h to k).

Overall, the 79%OA was the most stable oil treatment. The greater the 18:1 concentration, the greater the stability of the oil treatment, except that the greatly reduced 18:3 concentration in the LL treatment elevated its stability to be greater than or equal to that of the 37%OA, making it more stable than the control. Blending a poor stability oil, such as conventional SBO, with a high stability oil may had a profound effect only on the OSI and color of the blended oils but not on the other stability indicators.

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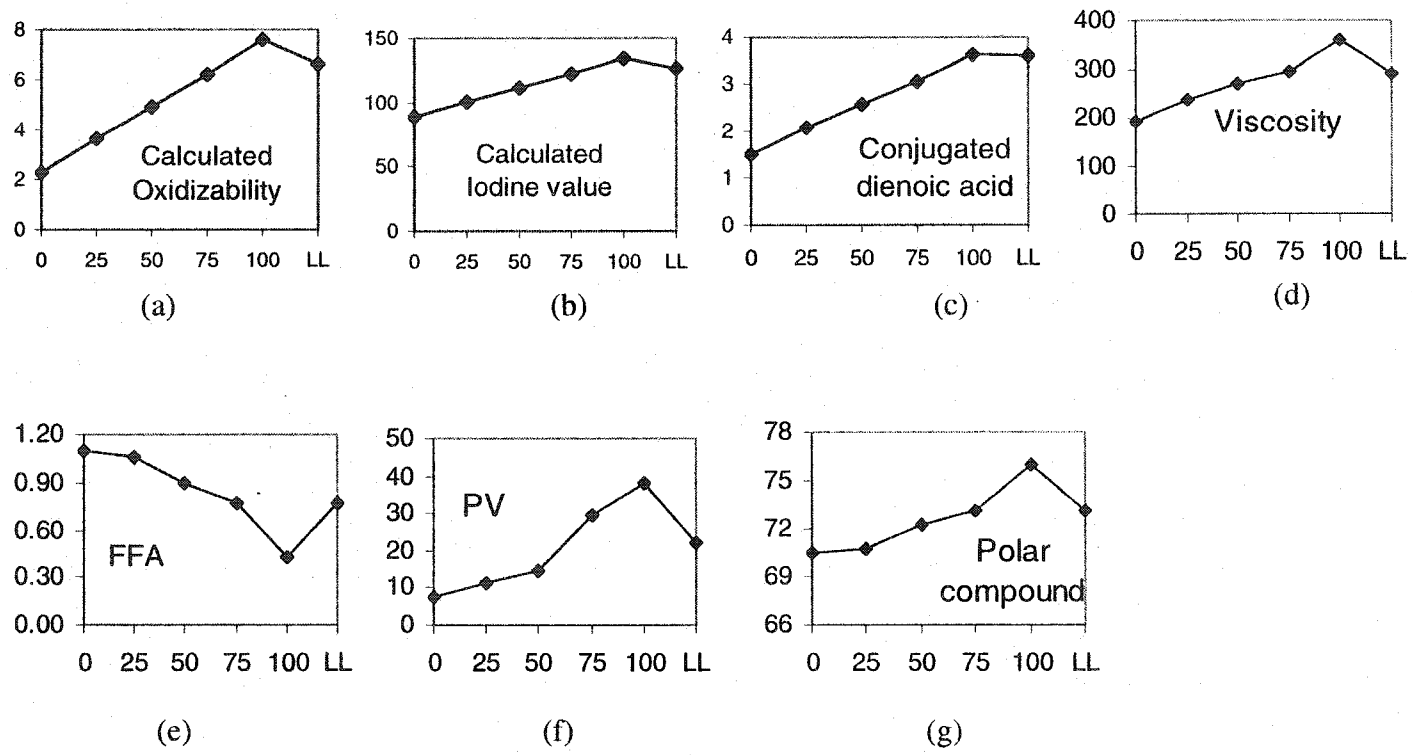
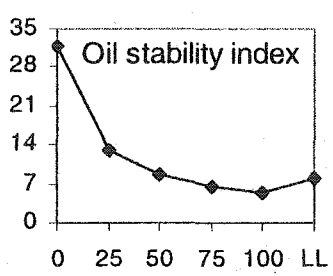


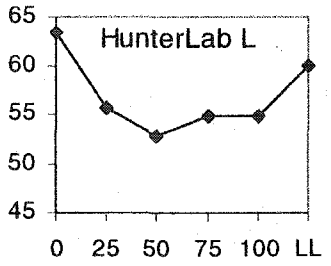
Figure 1. The impact of the % of the control present in the oil on the stability indices at 20 h heating.

The vertical axes are calculated oxidizability and iodine value, conjugated dienoic acid (%), viscosity (cP), FFA (% oleic acid), peroxide value (Meq/kg), polar compound (%), oil stability index (h), and HunterLab color values. The horizontal axis, 0, 25, 50, 75 representing the percentage of control (by weight) in the oil and LL representing the low-linolenic acid SBO treatment.

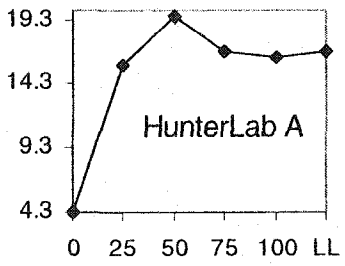
Figure 1. (continued)



(h)



(i)



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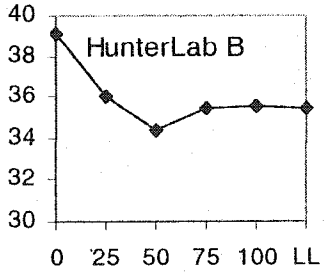

(k)

Table 1

FA Composition (area %), Calculated Oxidizability[a], Calculated Iodine Value[b], Tocopherols and Oil Stability Indices of Soybean Oil (SBO) Treatments

Oils[c]	Fatty Acid Methyl Esters					Oxidiz-	Iodine	Tocopherols (ug/g)[d]				OSI[e]
	16:0	18:0	18:1	18:2	18:3	ability	value	α	γ	δ	Total	
79%OA	6.9	3.8	79.0	6.5	3.8	2.3^{e}	89^{f}	113^{e}	722^{a}	495^{a}	1329^{a}	31.74^{a}
65%OA	7.8	3.9	64.7	18.7	4.9	3.6^{d}	101^{e}	156^{d}	722^{a}	$457^{a,b}$	1335^{a}	13.02^{b}
51%OA	9.0	4.1	50.7	30.3	6.0	4.9^{c}	112^{d}	199^{c}	722^{a}	$419^{b,c}$	1340^{a}	$8.63^{b,c}$
37%OA	9.9	4.3	36.9	41.8	7.1	6.2^{b}	123^{c}	242^{b}	723^{a}	$381^{d,c}$	1346^{a}	$6.48^{b,c}$
Control	11.2	4.4	21.5	54.8	8.0	7.6^{a}	134^{a}	285^{a}	723^{a}	343^{d}	1352^{a}	5.28^{c}
LL	10.6	4.5	25.3	58.2	1.4	6.6^{b}	126^{b}	274^{a}	731^{a}	286^{e}	1290^{a}	$8.13^{b,c}$

[a] Oxidizability = [oleate% + 10.3 (linoleate%) + 21.6 (linolenate%)]/100 (Ref. 9).

[b] Iodine values were calculated from the FAME profile, according to AOCS Official Method Cd 1c-85 (Ref. 12).

[c] 79.1%OA = high-oleic acid (OA) SBO. The 65%OA, 51%OA, 37%OA = three blends of control and 79%OA. Control = conventional SBO. LL = low-linolenic acid SBO.

[d] Tocopherol concentrations in 79%OA, Control, and LL SBO were determined. Tocopherol concentrations in the three blended oils were calculated.

[e] OSI = Oil stability indices.

Values in the same column for each test with supercripts in common were not significantly different ($p < 0.05$).

Table 2
FFA (% oleic), Viscosity (cP), Conjugated Dienoic Acid (%), Polar Compound (%), Hunter Lab Colors (L, a, b) and PV (meq/kg) of Frying SBOs[a] and Fried Bread Cubes

Analysis[b]	Soybean oil	Frying time (h) 0	Immediately after frying	10-h heating	20-h heating
FFA	79%OA	0.04^{a}	0.18^{a,b}	0.57^{a}	1.10^{a}
	65%OA	0.04^{a}	0.18^{a,b}	0.45^{b}	1.06^{a,b}
	51%OA	0.04^{a}	0.18^{a,b}	0.33^{c}	0.90^{b,c}
	37%OA	0.04^{a}	0.17^{a,b}	0.27^{c}	0.77^{c}
	Control	0.03^{a}	0.19^{a}	0.31^{c}	0.43^{d}
	LL	0.04^{a}	0.16^{b}	0.25^{c}	0.77^{c}
Viscosity[c]	79%OA	31.9^{a}	33.9^{a}	NA	189.9^{d}
	65%OA	29.1^{b}	32.1^{a,b}	NA	235.2^{c,d}
	51%OA	28.8^{b}	31.9^{a,b}	NA	271.0^{c}
	37%OA	27.4^{b,c}	32.2^{a,b}	NA	295.4^{b,c}
	Control	24.8^{d}	30.0^{b}	NA	358.2^{a,b}
	LL	26.7^{c,d}	29.3^{b}	NA	289.5^{b,c}
Conjugated	79%OA	0.10^{a}	0.44^{c}	0.97^{e}	1.49^{e}
Dienoic Acid	65%OA	0.10^{a}	0.65^{b,c}	1.69^{d}	2.07^{d}
	51%OA	0.10^{a}	0.84^{a,b}	2.34^{c}	2.57^{c}
	37%OA	0.10^{a}	1.09^{a}	2.90^{b}	3.06^{b}
	Control	0.10^{a}	1.15^{a}	3.41^{a}	3.62^{a}
	LL	0.10^{a}	0.91^{a,b}	3.33^{a}	3.60^{a}

[a] See footnote c in Table 1 for definitions of SBO treatments.

[b] Values in the same column for each test with supercripts in common were not significantly different ($p < 0.05$).

[c] Viscosity and colors of the oils at the end of first 10-h heating were not measured.

[d] Peroxide values of fresh SBOs used in frying, of SBOs extracted from fresh fried bread cubes, and of SBOs extracted from stored fried bread cubes.

Table 2 (continued)

Analysis[b]	Soybean oil	Frying time (h) 0	Immediately after frying	10-h heating	20-h heating
Polar Compounds	79%OA	1.9[a]	10.2[a]	47.5[c]	70.5[a]
	65%OA	1.9[a]	12.6[a]	55.7[b]	70.7[a]
	51%OA	1.6[a]	11.7[a]	53.9[b]	72.2[a]
	37%OA	1.8[a]	13.9[a]	53.8[b]	73.1[a]
	Control	2.0[a]	14.0[a]	67.4[a]	76.0[a]
	LL	2.2[a]	12.6[a]	62.5[a]	73.1[a]
HunterLab Color (L)[d]	79%OA	75.7[a]	72.2[c]		63.3[a]
	65%OA	75.5[a,b]	73.5[b]		55.7[b]
	51%OA	75.7[a]	73.7[b]		52.8[b]
	37%OA	75.9[a]	74.1[a,b]		54.7[b]
	Control	75.0[b]	74.3[a,b]		54.8[b]
	LL	75.2[a,b]	74.8[a]		59.8[a,b]
HunterLab Color (a)	79%OA	-2.4[a]	-5.4[a,b]		4.3[b]
	65%OA	-2.4[a]	-5.8[b]		15.7[a]
	51%OA	-2.5[a]	-5.8[b]		19.5[a]
	37%OA	-2.6[a]	-6.1[b]		16.8[a]
	Control	-2.4[a]	-5.6[a,b]		16.3[a]
	LL	-4.0[b]	-4.4[a]		16.8[a]
HunterLab Color (b)	79%OA	6.8[b]	24.3[a]		39.1[a]
	65%OA	6.5[b]	21.2[a,b]		36.0[b]
	51%OA	7.0[b]	20.0[a,b]		34.4[b]
	37%OA	7.2[b]	18.5[a,b,c]		35.4[b]
	Control	6.4[b]	16.5[b,c]		35.5[b]
	LL	12.0[a]	13.2[c]		35.4[b]
PV[d]		Fresh SBOs	Fresh bread		Stored bread
	79%OA	0.08[d]	5.45[b]		7.30[d]
	65%OA	0.10[d,c]	5.60[b]		11.37[d]
	51%OA	0.12[b,c]	5.60[b]		14.27[d,c]
	37%OA	0.15[a]	5.80[b]		29.47[a,b]
	Control	0.16[a]	6.60[a]		38.27[a]
	LL	0.14[a,b]	6.00[a,b]		22.03[b,c]

Optimizing Oleic Acid Composition of Soybean Oils for Best Flavor Stability and Quality during Frying

A paper to be submitted to the Journal of American Oil Chemists' Society

Caiping Su and Pamela White*

Department of Food Science and Human Nutrition and Center for Crops Utilization Research, Iowa State University, Ames, Iowa 50011

Running Title: FLAVOR STABILITY OF HIGH-OLEIC SOYBEAN OILS

* To whom correspondence should be addressed at 2312 Food Sciences Building, Iowa State University, Ames, IA 50011-1060. Phone: 515-294-9688, Fax: 515-294-8181, Email: pjwhite@iastate.edu

ABSTRACT: The objective of this project was to determine the optimum percentage of oleic acid (OA) in soybean oils (SBO) for maximum flavor stability and quality in fried foods. Six SBO treatments included a control (conventional SBO, 21.5% OA) and a high-oleic SBO (HO, 79% OA), which were tested as is. In addition, these two oils were blended in different ratios to make three blended oils containing 36.9%, 50.7%, and 64.7% OA, abbreviated as 37%OA, 51%OA, and 65%OA, respectively. Also, a low-linolenic (LL) SBO containing 1.4% linolenic acid and 25.3% OA was evaluated. White bread cubes of (8.19 cm^3) were fried in each of eighteen oils (6 treatments × 3 replicates). The fresh and stored bread cubes fried in 79%OA were second to the cubes fried in LL in overall flavor quality,

the weakest in intensity of stale, grassy, fishy, cardboard and burnt flavors by sensory evaluation, and contained the least amounts of hexanal, hexenal, t-2-heptenal, t,t-2,4-nonadienal, and t,t-2,4-decadienal in volatile analysis. Other treatments were intermediate in these sensory and instrumental evaluations, as related to their OA concentration. In general, the results suggested that the overall flavor stability and eating quality of foods fried in the six oil treatments from the best to the poorest would be: 79%OA ≥ LL, 65%-OA, 51%-OA, 37%-OA, and control.

KEY WORDS: Chernoff faces, fried bread cubes, frying oil stability, *high-oleic acid soybean oil*, low-linolenic acid soybean oil, sensory evaluation, volatile compound analysis.

Although soybean oil (SBO) has a good nutritional profile because of its high proportion of unsaturated FA, it has poor oxidative stability and is prone to flavor deterioration. The fatty acids, linoleic (18:2) and, especially, linolenic acid (18:3) in SBO, oxidize quickly and are the major contributors to the poor flavor stability of SBO (1, 2). Hydroperoxides formed by oxidation of 18:3 can break down to many undesirable flavor compounds, such as 2,4-heptadienal, 2-butylfuran, 2- and/or 3-hexenal, 2-pentenal and butanal (3). Hydroperoxides formed by oxidation of 18:2 can break down to undesirable flavor compounds, such as hexanal, under mild conditions and 2,4-decadienal at high temperatures (3).

To improve oxidative and flavor stability, SBO may be hydrogenated to reduce the concentration of PUFA (and increase the saturated FA); however, *trans* fatty acids (*t*FA) are formed during this process. Because of health concerns over the presence of *t*FA in our diets (4, 5), lowering the 18:3 content to a level similar to that obtained by partial hydrogenation,

but without *trans* formation, has been an objective of plant breeders. Soybean oils with different lowered levels of 18:3 have been developed and studied (6, 7). The flavor stability of SBO containing as low as 1.0% and 2.2% 18:3 was characterized by using a specialized program involving Chernoff faces in a previous study (7). The results showed that the former oil was more stable than the later. However, the 18:3 is an essential FA and belongs to a group called omega-3 FA, which have been shown to reduce or help prevent certain chronic diseases (8). Reducing 18:3 to a minimal level may diminish the health benefits of SBO. Also important to oxidation, is that the oxidation rate of OA is much slower than that of the PUFA, 18:2 and 18:3 (9). At the same time, a diet high in monounsaturates may help to reduce raised levels of total plasma cholesterol without reducing the HDL-cholesterol level (10). Therefore, developing SBO with enhanced stability and retained health benefits (low but not minimal 18:3, elevated oleic acid, no *t*FA, and minimal saturated FA) would be very desirable.

The overall objectives of this research, were to determine the optimum percentage of oleic acid (OA) in SBO for maximum flavor stability and eating quality in fried foods. Six oil treatments included a control (conventional SBO, 21.5% OA) and a high-oleic SBO (HO, 79% OA), which were tested as is. In addition, these two oils were blended in different ratios to make three blended oils containing 36.9%, 50.7%, and 64.7% OA, abbreviated as 37%OA, 51%OA, and 65%OA, respectively. Also, a low-linolenic (LL) SBO containing 1.4% linolenic acid and 25.3% OA was evaluated. A common belief is that blended oils are only as stable as the "poorest" oil present. Therefore, a secondary objective was to determine the impact of blending poor stability oil with high stability oil on the flavor and eating quality of the fried food.

MATERIALS AND METHODS

SBOs and design. Soybeans (*Glycine max*) with high-oleic acid (HO, 79% OA), low-linolenic acid (LL, 1.4% with 25.3% OA), and conventional (control, 21.3% OA) FA compositions, grown in summer 1998 in Iowa (weather zone 2), were obtained from Protein Technologies, Inc. (St. Louis, MO). The soybeans were crushed and the oils were hexane-extracted, in triplicate, in the Pilot Plant of the Center for Crops Utilization Research, Iowa State University (ISU), Ames, Iowa, following a previously published method (11). All the oils were refined and bleached following AOCS official methods Ca 9a-52, and Cc 8a-52, respectively, (12), and deodorized following the procedure described by Stone and Hammond (13). Triplicate sets of each oil were refined, bleached, and deodorized separately. Citric acid (100 ppm) was added to the oils during the cool-down stage of deodorization before placement in high-density polyethylene (HDPE) plastic bottles. The bottles were sparged with nitrogen, then sealed and stored at -10°C until used for testing.

Six SBO treatments were evaluated during frying, including the three SBO just mentioned (control, LL, and the 79%OA), plus three oil blends prepared as follows: 1) 75% of the control (by weight) and 25% of the HO (37%OA), 2) 50% of the control and 50% of the HO (51%OA), and 3) 25% of the control and 75% of the HO (65%OA).

Frying. Eighteen frying sessions (three simultaneous sessions in one day) were carried out (six oil treatments evaluated in triplicate). At each frying session, 220 g of an oil treatment was weighed into a Teflon-coated 473-mL electric baby fryer (National Presto Industries Inc., Eau Claire, Wisconsin) and the oil was then heated to 185°C within 10 min. The oil was heated at 185 ± 5°C for 2.5 h before frying. Eight 5-piece batches of crust-free

(finely ground with a spatula) from each sample was placed in a 20-mL vial and sealed. A 2-cm 50/30 um DVB/Carboxen/PDMS StableFlex fiber was inserted through the Teflon seal to trap the volatile compounds. The sealed sample was held at 40°C for 60 min, with the temperature maintained by a water bath on a hot plate. The extraction time was 60 min. The fiber was then removed from the vial and inserted into the injection port of a Hewlett Packard 5890 Series II GC equipped with a HP-5 30 m × 0.32 mm × 0.25 um column. The GC was programmed as follows: injection temperature 250°C, detector temperature 270°C, initial temperature 30°C, initial time 3 min, rate 4°C/min until reaching 100°C, then 8°C/min until reaching a final temperature of 220°C, which was held for 5 min. After injection, the fiber remained in the injection port for desorption for 10 min before being used for the next extraction. Individual external standards were used to identify retention times for each flavor compound found in the bread cubes. For this procedure, a volume of 0.5 μl standard was injected into the fried bread cube (about 3.0 g, ground as previously discribed) with a syringe inserted through the Teflon seal. The vial was shaken and the rest of the steps were the same as just described.

Statistical analysis. There were 6 treatments × 3 replicates. The SAS general linear model procedure (Program GLM) was used to analyze the data (18). Differences in mean values among treatments were determined by the least significant difference test at $\alpha = 0.05$, unless listed otherwise.

Statistical software S-plus 6.0.3 Release 2 for Microsoft Windows was used to draw the faces representing sensory characteristics of fried bread cubes. Another paper (7) described in detail the application of Chernoff faces to the data analysis of sensory evaluation of food

bread cubes (2.54 cm x 2.54 cm x 1.27 cm) were fried for 1 min per batch at 3-min intervals. Therefore, the actual frying of the cubes was completed within 0.5 h. The fried bread cubes were then drained and cooled to room temperature. Half of the bread cubes was used immediately for testing, including evaluating flavor characteristics by a trained sensory panel and instrumental volatile analysis by GC-SPME method. The other half of the bread cubes was stored, loosely covered, at 60°C in the dark for 3 days before sensory evaluation and volatile analysis by the same procedures used on fresh bread cubes. The oil remaining in the fryer was maintained at 185 ± 5°C for another 7 h for a total of 10 h heating on day 1, then cooled to 25°C. The oil was heated at 185 ± 5°C for another 10 h on day 2.

Fatty acid composition by GC, tocopherol contents by HPLC. Fatty acid compositions of SBO before frying were determined according to a method described by Hammond (14). The GC conditions were the same as described by Shen *et al.* (11). Tocopherol contents were determined according to AOCS Official Method Ce 8-89 (12). The HPLC conditions were the same as described elsewhere (6).

Sensory evaluations of the fried bread cubes. Sensory evaluations were conducted according to AOCS Recommended Practice Cg 2-83 (12). A 12-member trained descriptive panel was used to evaluate overall flavor quality and individual flavor and off-flavor intensities of the fried bread cubes. All panelist candidates (17 members) were trained during four 1-h sessions. During training, panelists were given definitions for 10 flavor descriptors, including fried food, cardboard, waxy, stale, grassy, burnt, acrid, fishy, rancid, and painty flavors (15). Standards for these 10 flavors, respectively, included fresh French fries from a local fast-food restaurant, water with cardboard soaked in it for 1 h, melted paraffin oil, potato chips aged 2 weeks at room temperature, fresh-cut green grass, burned fried bread

cubes, canola oil heated to 240°C for 5 min, canola oil heated to 190°C for 3 min, SBO with a PV of 18 meg/kg and canola oil kept at room temperature for 3 years (15). Candidates were asked to smell or taste the standards and to assign an intensity score. Also, candidates were given fresh SBO, SBO with a PV of 18 meg/kg, and canola oil kept at room temperature for 3 years to smell and rank in order of painty intensity. Candidates who incorrectly ordered the intensity of painty flavor in these samples or could not detect flavors from the ten standards, after training, were omitted (5 out of 17 people) as panelists.

For the actual tests, in each session, three bread cubes from three different treatments were presented to each panelist. The cubes were presented on paper plates, labeled with random, three-digit codes, and presented in random order to panelists. Panelists were asked to smell the cubes first, then bite into the bread to taste. To avoid tasting fatigue and flavor carry-over, panelists were given only three samples per session, and were asked to expectorate the sample after tasting and to rinse their mouths with distilled water between tasting samples. Evaluations were conducted in 12 individual, lighted booths. The breads were evaluated for overall flavor quality on a 10-point scale (10=excellent quality, 9 and 8=good, 7 and 6=fair, 5 and 4=poor, 3, 2, and 1=very poor) and for intensity of the 10 individual flavors listed in the previous paragraph on a 10-point scale (10=bland, 9=trace, 8=faint, 7=slight, 6=mild, 5=moderate, 4=definite, 3=strong, 2=very strong, 1=extreme). Overall flavor quality scores were calculated as the average of all overall quality scores given by the panelists. Intensity of a flavor was calculated as the average of the intensity scores by the panelists who detected the flavor in the sample.

Volatile profile of the bread cubes by GC-SPME. The procedures by Jelen *et al* (16) and Roberts *et al* (17) were followed with some modifications as described. About 3.0 g bread

products, especially oils. Each sensory attribute of the fried bread was assigned to a facial feature (Table 1) enabling a cartoon of a face to be drawn by using the data obtained from the sensory panel. For example, the overall quality of the bread cubes from 10 to 1 determined the size of the face from large to small, the stale intensity from 10 to 1 determined the length of the nose from long to short, and so on.

RESULTS AND DISCUSSION

Fatty acid composition (Table 2). The control oil had much greater palmitic (16:0), 18:2, and 18:3 acid concentrations than did the 79%OA. The blended treatments were intermediate in these FA levels, based on the ratios of each oil percent present. The LL was similar in FA composition to the control, except for its greatly reduced 18:3 level, and slightly increased 18:1 and 18:2 levels.

Tocopherols (Table 2). There were no differences in the concentrations of total tocopherol concentrations among 79.1%OA, control and LL SBO, and any of their blends (19).

Sensory evaluations of the fried bread cubes (Table 3). The fresh and stored bread cubes of the LL treatment generally had the best overall flavor quality, the 79%OA the second, the control the worst, and the three blended treatments were intermediate, based on their OA concentrations, but the differences were not statistically significant.

Among all fresh fried bread cubes, the 79%OA tended to have the weakest fishy, cardboard (same as 51%OA and LL) and burnt (same as LL) flavors, was second weakest to LL (same as control) in stale flavor, and second weakest behind 65%OA and 37%OA (the same) in grassy flavor. LL tended to have the weakest rancid, cardboard (same as 51%OA and 79%OA), acrid and burnt (same as 79%OA) flavor, was second weakest after 65%OA

and 37%OA (the same) in grassy flavor, second weakest to 79%OA in fishy flavor, and second weakest after 51%OA and 37%OA (the same) in painty flavor. In general, the LL fresh fried bread cubes had the best flavor characteristics among all fresh treatments followed by 79%OA. The control generally had the most intense grassy, fishy, acrid, and burnt flavors.

In general, among the stored fried bread cubes, the 79%OA was the weakest in fried food (the same as control), stale (the same as control), grassy, and burnt flavors, was second weakest along with LL behind 51%OA in fishy flavor and second weakest behind 65%OA and LL (the same) in acrid flavors. The LL had the most intense fried food flavor, had the weakest waxy, cardboard (the same as 65%OA), and acrid (the same as 65%OA) flavors, was the same as 65%OA and second weakest behind 79%OA in stale flavor, was the same as 79%OA and second weakest behind 51%OA in fishy, and was the same as control and 79%OA and the second weakest behind 65%OA in painty flavors. The control tended to have the weakest fried food (the same as 79%OA), and the most intense grassy (the same as LL) and burnt flavors.

The 18:3 in SBO is known as the major contributor of poor flavor stability (1, 2). The above results of fresh and stored bread cubes demonstrated that the greatly reduced 18:3 in LL SBO greatly elevated its flavor stability and quality over those of other treatments that contained more 18:3. The greatly increased 18:1 in 79%OA likely improved its flavor quality over that of other treatments as demonstrated by weaker stale, grassy, fishy, and cardboard flavors of the food fried in it. However, the fresh and stored cubes fried in 79%OA tended to have weaker fried food flavor than the blended oils that contained a fair amount of 18:2, the FA proposed to generate fried food flavor during frying (3). The inconsistency to this reasoning is that both the fresh control and the LL treatments, having the greatest amount of

18:2, have even weaker fried food flavor than the 79%OA treatment. After storage, the control continued to have the weakest fried food flavor, but the LL treatment tended to have the strongest fried food flavor among all stored treatments. Perhaps interactions among flavors when bread cubes were fresh and when the treatments were more prone to flavor deterioration decreased the intensity of fried food flavors to panelists.

Chernoff faces were used to represent the overall sensory characteristics of the fresh and stored bread cubes (Fig 1). Each sensory attribute of the fried bread was assigned to a facial feature (Table 1) enabling a cartoon of a face to be drawn from the data obtained from the sensory panel. For example, the overall quality of the bread cubes from 10 to 1 determined the size of the face from large to small, respectively, the stale intensity from 10 to 1 determined the length of the nose from long to short, respectively, and so on. Glancing at Fig 1, one can see among the fresh fried bread cubes, the treatments of LL and 79%OA created similar overall sensory perceptions, but LL tended to have a slightly better overall quality score (larger face). The treatments of 65%OA, 51%OA and 37%OA had overall sensory perception similar to each other. The control tended to be most different in overall sensory perception from the other treatments.

The trend of overall sensory characteristics of stored bread cubes were generally the same as that of the fresh bread cubes. The LL and 79%OA still were similar in overall sensory perception. The 65%OA, 51%OA and 37%OA were somewhat similar to each other, and the control was most different from other treatments in overall sensory perception.

Volatile profile of the fried bread cubes by GC-SPME. Both the fresh and stored fried cubes of 79%OA treatment had significantly less hexenal and less t,t-2,4-heptadienal, although not significantly, than did those of control. The three blends were intermediate

between the 79%OA and control and were generally not different from each other for the concentration of these two volatiles. When fresh, the LL bread had significant less amount of hexenal than did that of control, significantly less amount of t,t-2,4-heptadienal than did that of the other treatments (Figure 2a). After storage, the LL bread had significant less amount of hexenal than did that of control and 65%OA, less but not significant amount of t,t-2,4-heptadienal than did that of the other treatments (Figure 2b). Oxidation of 18:3 is known to produce 2,4-heptadienal and hexenal (3, 19). Fair positive correlation between the amounts of these two compounds in fried bread cubes and the concentration of 18:3 in the corresponding frying oils were found (Table 4). There was also fair positive correlation between the amount of hexenal in fresh fried bread cubes and the concentration of 18:2 in the corresponding frying oils. The fresh and stored control and LL bread cubes generally had more hexanal, t-2-heptenal, t,t-2,4-nonadienal, and t,t-2,4-decadienal than the 79%OA and the differences were generally significant, except for hexanal (Figure 2). The fresh and stored bread cubes of the three blends had concentrations of these compounds that were intermediate between 79%OA and the control and LL, and related to the 18:2 concentration of the corresponding frying oils. There were strong positive correlation coefficients between the production of these compounds in the fried bread cubes and the concentration of the 18:2 of the frying oils, except for hexanal. This relationship can be explained by the finding that oxidation of 18:2 favored enals and dienals at higher temperatures. Although hexanal is a breakdown product of 18:2, its formation is favored under mild conditions, thus its poor correlations were not surprising (20, Table 4). The 79%OA tended to produce more nonanal and t-2-decenal than the other treatments and there were strong positive correlation between

the amounts of these two compounds in the fresh and stored fried bread cubes and the initial concentration of 18:1 in the corresponding frying oils (Table 4).

The compounds noted in Figure 2 may play significant roles in flavor characteristics of food, because of their low thresholds and specific flavor characteristics (19, 21). Previous studies estimated the significance of some volatile compounds from the oxidation of soybean oil on food flavor, based on their concentrations and threshold values. *Trans*, *cis*-2,4-decadienal was the most flavorful followed by *trans*, *trans*-2,4-decadienal, *trans*, *cis*-2,4-heptadienal, 1-octen-3-ol, n-butanal, n-hexanal, *trans*, *trans*-2,4-heptadienal, 2-heptenal, n-heptanal, n-nonanal, and 2-hexenal (22). In the current study, the greater amount of hexanal (fresh fried bread cubes), *trans*, *trans*-2,4-heptadienal (fresh), and hexenal (fresh and stored fried bread cubes) present in the control may have contributed to its strong grassy and fishy off-flavors. Conversely, the generally low amounts of these compounds may have resulted in grassy and fishy off-flavors in 79%OA and LL. The tendency for more t-2-heptenal and t,t-2,4-decadienal to be present in the control and LL treatments may have caused slightly stronger rancid and fried food flavor in the fresh fried cubes.

There were strong positive correlations between the amounts of nonanal and t-2-decenal and the 18:1 concentration, which may explain the stale, waxy-like off-flavor sometimes associated with high oleic acid SBO. Nonanal was previously described as tasting fruity and t-2 decenal was described as tasting plastic (23). However, it is still controversial about what compounds cause what particular flavors in fats and oils for two reasons. On one hand, it is difficult to agree on the common terms to describe the same odor or off-flavor by different researchers. On the other hand, little progress has been made in relating flavor descriptors

with individual volatile compounds in a natural mixture, such as food, due to additive and antagonistic interactions between volatile compounds (24).

Overall, the 79%OA had better flavor stability and quality than did the control. But, the impact of 18:1 elevation on flavor stability was not as pronounced as that on its oxidative stability reported in a related paper as measured by peroxide value, FFA, conjugated dienoic acid, polar compound percentage, and viscosity of the frying oils (19).

The greatly reduced 18:3 concentration in the LL treatment elevated its flavor stability and quality to be equal to or greater than that of the 79%OA, greater than that of the blends and much greater than that of the control. The impact of reducing 18:3 concentration on flavor stability was greater than that on the oxidative stability (19), likely because of the significance of the volatiles (*trans*, *trans*-2,4-heptadienal and hexenal) produced from breakdown of 18:3. In the oxidative stability tests, LL was only equivalent to 37%OA. These findings further demonstrated that 18:3 is a major contributor of flavor instability in SBO.

The impact of blending poor stability oil with high stability oil on flavor quality and stability of the three blends was profound that the three blends had stronger off-flavor such as stale, fishy, and burnt than did those of 79%OA but also stronger favorable fried food flavor, which maybe explained by the fact that the blends had fair amounts of 18:1, 18:2 and 18:3 fatty acids that oxidize to both favorable and unfavorable flavor compounds. A good balance of all these flavor compounds provides good flavor quality for the food. Therefore, a balanced fatty acid composition in the blends may result in good flavor quality and characteristics of the blends.

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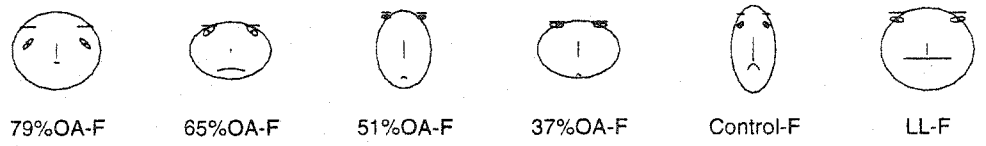


Figure 1a. Sensory Characteristics of Fresh Fried Bread Cubes[a].

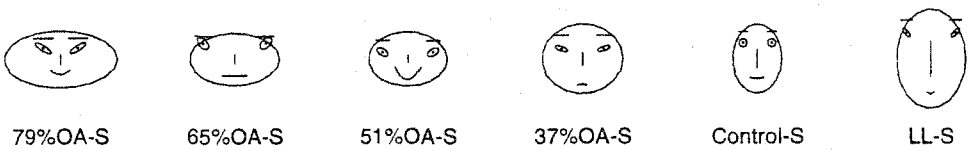


Figure 1b. Sensory Characteristics of Stored Fried Bread Cubes.

[a]Refer to footnote a of Table 2 for treatment definitions of 79%OA, 65%OA, 51%OA, 37%OA, Control, LL. The "-F" refers to fresh fried bread cubes and the "-S" refers to stored fried bread cubes.

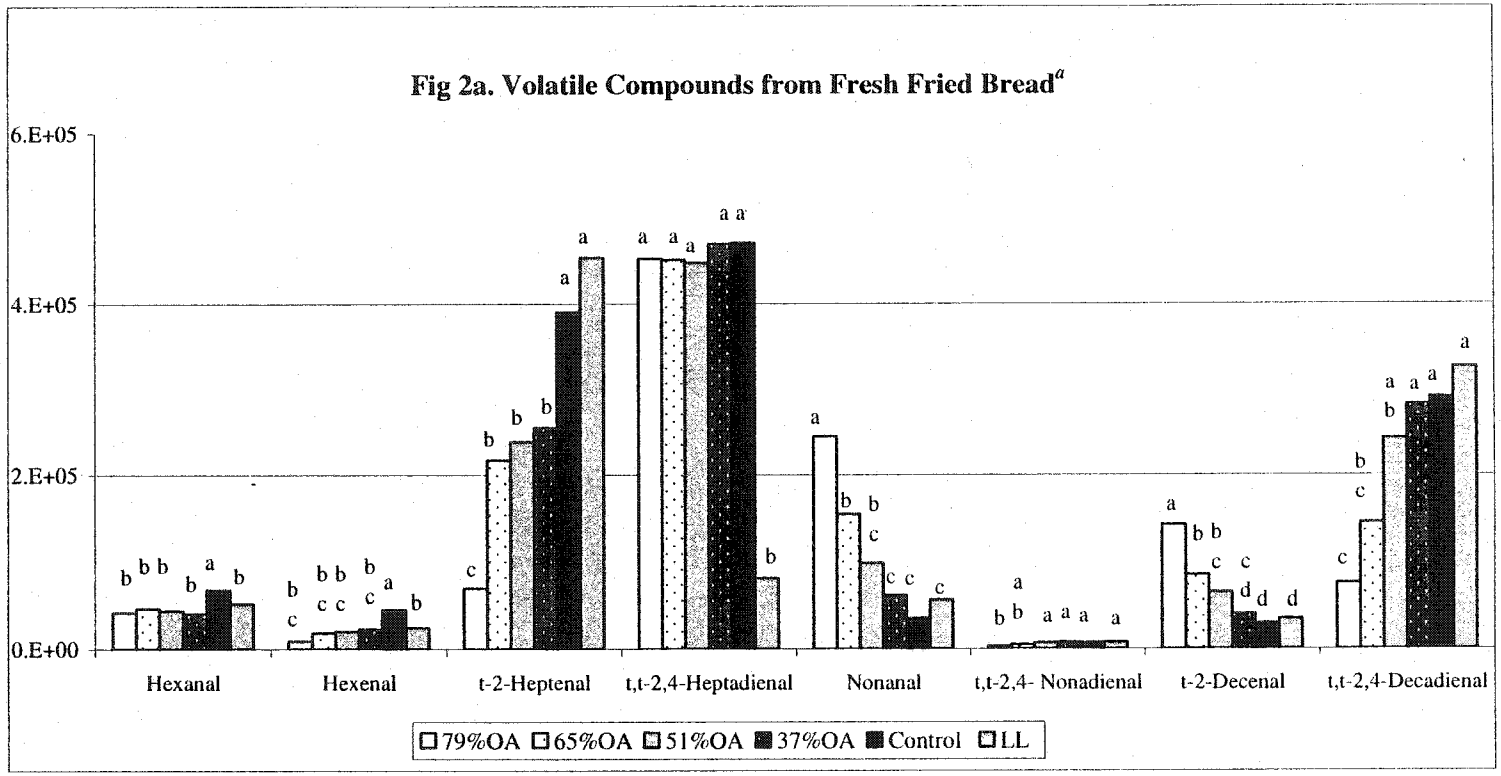
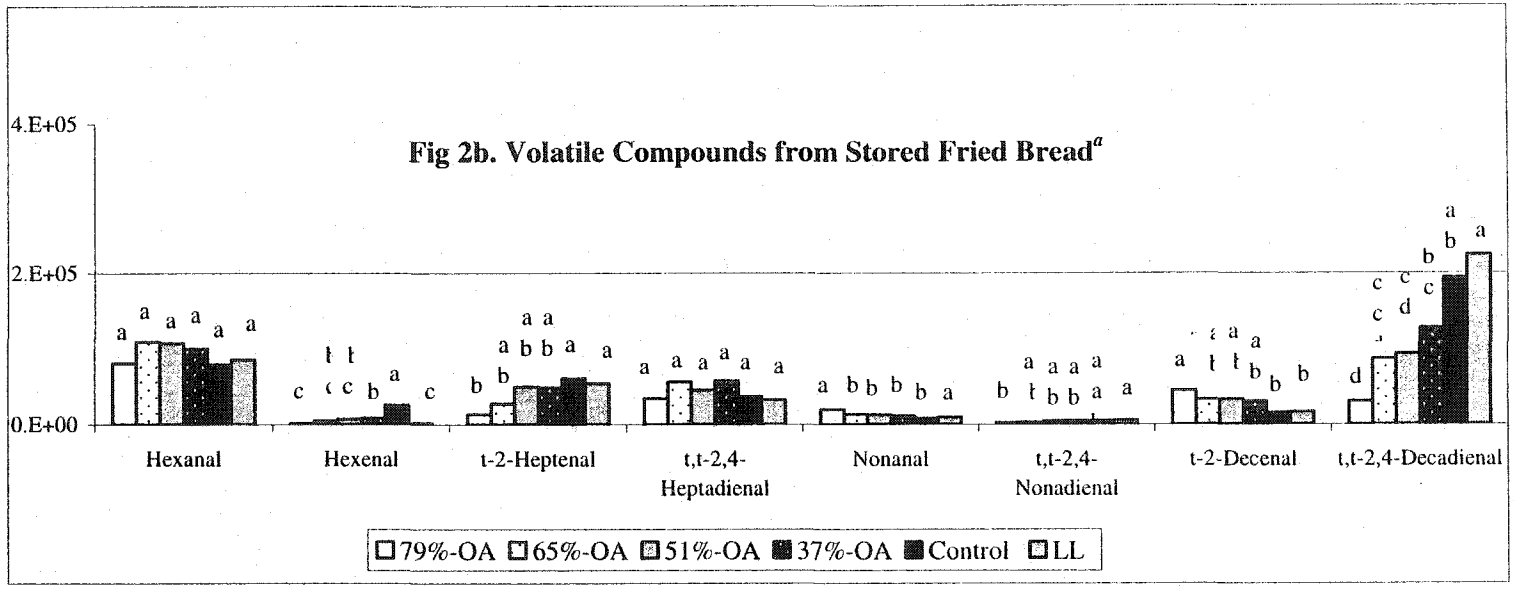


Fig 2a. Volatile Compounds from Fresh Fried Bread[a]
6.E+05
4.E+05
2.E+05
0.E+00
Hexanal
Hexenal
t-2-Heptenal
t,t-2,4-Heptadienal
Nonanal
t,t-2,4- Nonadienal
t-2-Decenal
t,t-2,4-Decadienal
79%OA
65%OA
51%OA
37%OA
Control
LL



[a]For each volatile compound, values with label letters in common were not significantly different ($p < 0.05$).

Table 1
Correspondence between the Assigned Facial Features and the Flavor Descriptors of the Fried Bread Cubes

Dimension	Flavor attribute	Facial feature	Numerical value assigned
1	Overall quality	Area of face	10-1 from sensory data
2	Grassy	Shape of face	10-1 from sensory data
3	Waxy	Length of nose	10-1 from sensory data
4	Stale	Location of mouth	10-1 from sensory data
5	Fishy	Curve of smile	10-1 from sensory data
6	Rancid	Width of mouth	10-1 from sensory data
7	Painty	Location of eyes	10-1 from sensory data
8	Cardboard	Separation of eyes	10-1 from sensory data
9	Acrid	Angle of eyes	10-1 from sensory data
10	Burnt	Shape of eyes	10-1 from sensory data
11	-	Width of eyes	5
12	-	Location of pupil	5
13	-	Location of eyebrow	5
14	-	Angle of eyebrow	5
15	-	Width of eyebrow	5

[a] The sign "-" means no flavor attribute was assigned to that facial feature and S-plus assumes a mid-value of "5" to that feature to draw a complete face.

[b] Value of 10 (excellent/bland) to 1 (very poor/extremely strong) were given to each flavor characteristic of oils, according to the panelists' scores.

Table 2
FA Composition (area %) and Tocopherols of Soybean Oils (SBO)

Oil[a] Treatments	Fatty Acid Methyl Esters					Tocopherols (ug/g)[b]			
	16:0 (palmitic)	18:0 (stearic)	18:1 (oleic)	18:2 (linoleic)	18:3 (linolenic)	α	γ	δ	Total
79%OA	6.9	3.8	79.0	6.5	3.8	113^{e}	722^{a}	495^{a}	1329^{a}
65%OA	7.8	3.9	64.7	18.7	4.9	156^{d}	722^{a}	$457^{a,b}$	1335^{a}
51%OA	9.0	4.1	50.7	30.3	6.0	199^{c}	722^{a}	$419^{b,c}$	1340^{a}
37%OA	9.9	4.3	36.9	41.8	7.1	242^{b}	723^{a}	$381^{d,c}$	1346^{a}
Control	11.2	4.4	21.5	54.8	8.0	285^{a}	723^{a}	343^{d}	1352^{a}
LL	10.6	4.5	25.3	58.2	1.4	274^{a}	731^{a}	286^{e}	1290^{a}

[a] 79%OA = high oleic-acid (OA) SBO. The 65%OA, 51%OA, 37%OA = three blends containing % of OA indicated, achieved by blending 79%OA with the control SBO.
LL = the low linolenic acid SBO.

[b] Values in the same column for each test with supercripts in common were not significantly different ($p < 0.05$).

Table 3

The Flavor Characteristics[a] of Fresh and Stored Fried Bread Cubes by Sensory Evaluations[b]

Fried Bread Cube Treatments[c]		Overall flavor quality[c]	Fried food	Stale	Waxy	Grassy	Fishy	Rancid	Painty	Card-board	Acrid	Burnt
Fresh	79%-OA	6.6	4.1	9.7	9.3	9.7	9.3	8.8	9.0	9.5	8.3	9.3
	65%-OA	6.1	4.0	9.0	9.0	9.8	9.0	9.3	9.5	9.3	8.4	8.7
	51%-OA	6.0	3.8	9.0	9.4	9.5	8.8	8.9	9.6	9.5	9.0	8.6
	37%-OA	6.1	3.8	8.7	9.5	9.8	8.6	8.8	9.6	9.3	9.0	8.9
	Control	5.9	4.4	9.8	9.5	9.3	8.4	9.2	9.3	9.4	8.3	8.1
	LL	6.8	4.7	9.8	9.2	9.7	9.1	9.6	9.5	9.5	9.1	9.3
Stored	79%-OA	6.5	5.0	9.3	9.3	10.0	9.4	9.0	9.2	8.9	9.5	9.3
	65%-OA	6.1	4.2	9.1	9.3	9.9	9.1	9.2	9.4	9.6	9.7	8.1
	51%-OA	6.0	4.6	8.8	9.2	9.8	9.8	9.3	9.1	9.5	9.5	8.2
	37%-OA	6.3	4.3	8.9	9.3	9.7	9.0	8.9	9.1	9.3	9.3	8.9
	Control	5.8	5.0	9.3	9.5	9.5	9.2	9.2	9.2	9.4	9.4	7.6
	LL	6.6	4.1	9.1	9.7	9.5	9.4	8.9	9.2	9.6	9.7	8.8

[a] Values obtained from sensory panels. For overall flavor quality, 10 = excellent, 1 = very poor. For the intensity of individual flavors, 10 = bland, 1 = very strong.

[b] Values in the same column were not significantly different ($p < 0.05$) for fresha nd stored treatments, respectively.

[c] See footnote [a] of Table 2 for treatment abbreviations.

Table 4
Correlation Between the Mean Amount of Individual Volatile Compounds from Fried Bread Cubes and the Mean Concentration of a Specific FA in the Correponding Frying Oils

Volatile compound	Fatty acid	Fresh fried bread cubes		Fresh fried bread cubes	
		Correlation coefficient	ρ value	Correlation coefficient	ρ value
Hexenal	18:3	0.536	0.279	0.779	0.070
	18:2	0.785	0.064	0.496	0.317
	18:1	-0.835	0.039	-0.580	0.227
t,t-2,4-Heptadienal	18:3	0.807	0.051	0.462	0.354
	18:2	-0.512	0.299	-0.209	0.693
	18:1	0.408	0.430	0.152	0.773
Hexanal	18:3	0.269	0.615	0.174	0.742
	18:2	0.632	0.176	-0.274	0.601
	18:1	-0.653	0.159	0.251	0.630
t-2-Heptenal	18:3	-0.069	0.887	0.338	0.520
	18:2	0.957	0.003	0.929	0.007
	18:1	-0.932	0.007	-0.953	0.003
t,t-2,4-Nonadienal	18:3	0.274	0.606	0.062	0.914
	18:2	0.904	0.013	0.941	0.005
	18:1	-0.920	0.009	-0.931	0.007
t,t-2,4-Decadienal	18:3	0.141	0.797	-0.062	0.899
	18:2	0.965	0.002	0.973	0.001
	18:1	-0.964	0.002	-0.949	0.004
Nonanal	18:3	-0.332	0.526	-0.278	0.602
	18:2	-0.942	0.005	-0.927	0.008
	18:1	0.964	0.002	0.943	0.005
t-2-Decenal	18:3	-0.289	0.586	-0.065	0.912
	18:2	-0.941	0.005	-0.954	0.003
	18:1	0.958	0.003	0.944	0.005

GENERAL CONCLUSIONS

Overall, this study demonstrated 1): the importance of 18:3 concentration of the oil to its oxidative and flavor stability. Reducing 18:3 concentration can greatly improve flavor quality of the oil and of fried food in the oil. During storage under fluorescent light at both 21°C and 32°C, the SBO with ultra-low-18:3 concentration (1.0%, ULSBO) generally had greater oxidative stability than did SBO with low-18:3 concentration (2.2%, LLSBO). Although the ULSBO initially had significantly greater initial oxidation (greater peroxide values and poorer (lower) sensory scores for overall flavor quality) than did LLSBO, significant differences disappeared with storage and the ULSBO was indeed more stable than LLSBO. Among the six oil treatments used in frying, the low-linolenic (LL) had oxidative stability slightly better than the conventional SBO (Control) and equivalent to that of the 37%-OA SBO (blended SBO containing 37% oleic acid), but the flavor quality of the food fried in the LL was the best;

2): elevating OA in vegetable oil greatly improves its oxidative stability. However, the effect on the flavor quality was not as obvious as that on the oxidative stability. Among the six oil treatments used in frying, the 79%-OA (natural HO SBO with 79% olec acid) was the most oxidatively stable one, but the food fried in it had significantly greater amount of nonanal and *t*-2-decenal than other treatment. Hammond (personal communication) indicated that the contribution of these two compounds to the unique "stale", "waxy" off-flavor sometimes associated with HO SBO may have been overlooked. Sensory evaluation of the

bread cubes fried in the 79%-OA SBO was weak in fried food flavor and it was not the best for overall flavor quality as it was the most oxidatively stable one among the six treatments.

In the future, it will be interesting and important to study the performance of the low-linolenic and high-oleic SBO in commercial applications, as it is always the initial purpose of developing new soybeans with enhanced properties.

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